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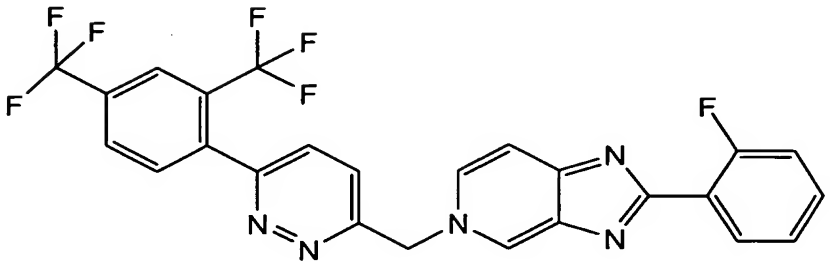
(54) Title: CRYSTALLINE PYRIDAZINE COMPOUND



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(57) Abstract: A crystalline compound of formula (1) and its salts and solvates are provided for the treatment or prophylaxis of hepatitis C virus infections (1) Methods of making and formulating crystalline compound (1) are provided.

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CRYSTALLINE PYRIDAZINE COMPOUND

Background of the Invention

The hepatitis C virus is an enveloped, single-stranded, positive sense RNA virus in the family *Flaviviridae*. HCV mainly replicates within hepatocytes in the liver. Circulating HCV particles bind to receptors on the surfaces of hepatocytes and subsequently enter the cells. Once inside the hepatocyte, HCV utilizes the intracellular machinery necessary to accomplish its own replication. Lindenbach, B. Nature 436(7053):932-8 (2005). The HCV genome is translated to produce a single protein of around 3011 amino acids. This "polyprotein" is then proteolytically processed by viral and cellular proteases to produce three structural (virion-associated) and seven nonstructural (NS) proteins.

HCV encodes two proteases, the NS2 cysteine autoprotease and the NS3-4A serine protease. The NS proteins then recruit the viral genome into an RNA replication complex, which is associated with rearranged cytoplasmic membranes. RNA replication takes places via the viral RNA-dependent RNA polymerase of NS5B, which produces a negative-strand RNA intermediate. The negative strand RNA then serves as a template for the production of new positive-strand viral genomes. Nascent genomes can then be translated, further replicated, or packaged within new virus particles. New virus particles presumably bud into the secretory pathway and are released at the cell surface.

HCV has a high rate of replication with approximately one trillion particles produced each day in an infected individual. Due to lack of proofreading by the HCV RNA polymerase, HCV also has an exceptionally high mutation rate, a factor that may help it elude the host's immune response.

Based on genetic differences between HCV isolates, the hepatitis C virus species is classified into six genotypes (1-6) with several subtypes within each genotype. Subtypes are further broken down into quasispecies based on their genetic diversity. The preponderance and distribution of HCV genotypes varies globally. For example, in North America genotype 1a predominates followed by 1, 2a, 2b, and 3a. In Europe genotype 1 is predominant followed by 2a, 2b, 2c, and 3a. Genotypes 4 and 5 are found almost exclusively in Africa. Genotype is clinically important in determining potential response to interferon-based therapy and the required duration of such therapy. Genotypes 1 and 4 are less responsive to interferon-based treatment than are the other genotypes (2, 3, 5 and 6). Duration of standard interferon-based therapy for genotypes 1 and 4 is 48 weeks, whereas treatment for genotypes 2 and 3 is completed in 24 weeks.

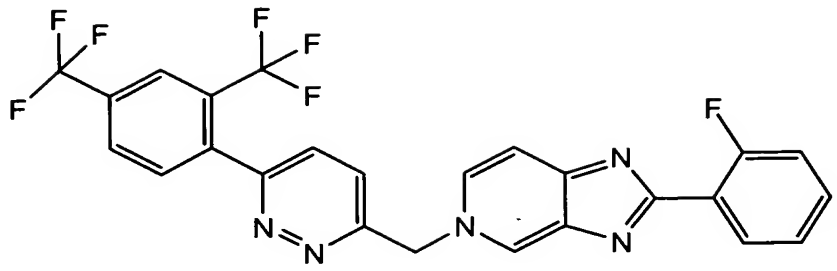
The World Health Organization estimates that world-wide 170 - 200 million people (3% of the world's population) are chronically infected with HCV. Approximately 75% of these individuals are chronically infected with detectable HCV RNA in their plasma. These chronic carriers are at risk of developing cirrhosis and/or liver cancer. In studies with a 7-16 years follow-up, 7-16 % of the patients developed cirrhosis, 0.7-1.3% developed hepatocellular carcinoma and 1.3-3.7% died of liver-related disease.

The only treatment option available today is the use of interferon α-2 (or its pegylated form) either alone or combined with ribavirin. However, sustained response is only observed in about 40% of the patients and treatment

is associated with serious adverse effects. There is thus an urgent need for potent and selective inhibitors of HCV.

Relevant disclosures include U.S. Patent Nos. 4,914,108; 4,988,707; 4,990,518; 5,137,896; 5,208,242; 5,227,384; 5,302,601; 5,374,638; 5,405,964; 5,438,063; 5,486,525; 6,479,508; and U.S. Patent Publication No. US2003/0108862 A1, Canadian Patent No. 2423800 A1, German Patent Nos. 4211474 A1, 4236026, 4309969, 4318813, European Patent Nos. EP 0 138 552 A2, EP 0 706 795 A2, EP 1 132 381 A1, Great Britain Patent No. 2158440 A, PCT Patent Publication Nos. WO 00/20416, WO 00/39127, WO 00/40583, WO 03/007945 A1, WO 03/010140 A2, WO 03/010141 A2, WO 93/02080, WO 93/14072, WO 96/11192, WO 96/12703, WO 99/27929, PCT-US2004/43112, PCT-BE2003/000117, PCT-US2005/26606, Akamatsu, et al., "New Efficient Route for Solid-Phase Synthesis of Benzimidazole Derivatives", 4:475-483, *J. COMB. CHEM.*, 2002, Baginski SG et al., Proc. Natl. Acad. Sci. U.S.A. 2000 Jul 5;97(14):7981-6). Cleve et al., "Derivate des Imidazo[4.5-b]- und Imidazo[4.5-c]pyridins", 747:158-171, *JUSTUS LIEBIGS ANNALEN DER CHEMICA*, 1971, Kiyama, et al., "Synthesis and Evaluation of Novel Nonpeptide Angiotensin II Receptor Antagonists: Imidazo[4,5-c]pyridine Derivatives with an Aromatic Substituent", 43(3):450-60, *CHEM PHARM BULL*, 1995, Mederski et al., "Synthesis and Structural Assignment of Some N-substituted Imidazopyridine Derivatives", 48(48):10549-58, *TETRAHEDRON*, 1992, Yutilov et al., 23(1):56-9, *KHIMIKO-FARMATSEVTICHESKII ZHURNAL*, 1989. In addition, see WO 05/063744.

The compound of formula (1) is the subject of WO 08/005519.



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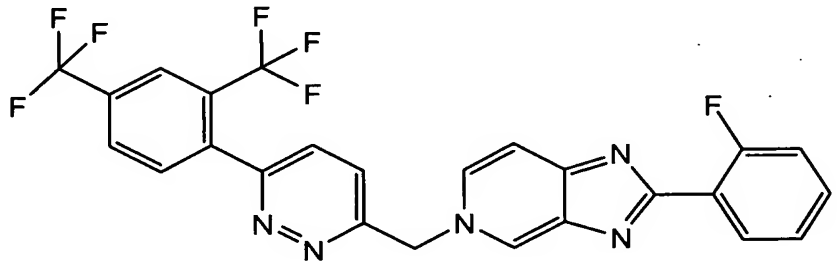
Compound (1) as produced by the process of WO 05/063744 is substantially or entirely amorphous. It is believed to be a hydrate (hereafter "amorphous" compound (1)).

It is an object of this invention to provide compound (1) in crystalline form.

Summary of the Invention

In accordance with achieving the foregoing objects of this invention, a crystalline compound is provided having formula (1)

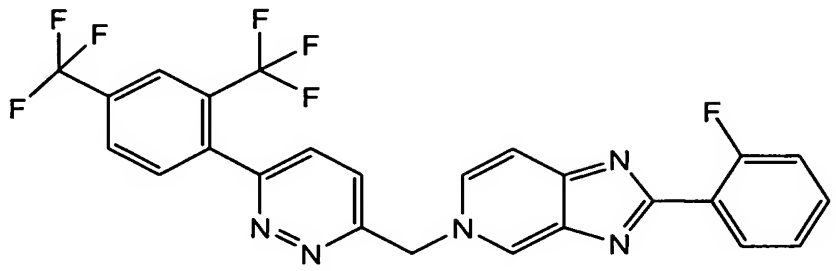


(1)

and its salts, which is substantially free of amorphous compound (1) .

In an embodiment, the crystalline compound (1) is the free base substantially free of amorphous compound (1) and any other crystal form of compound (1).

Another embodiment of this invention is a method for making crystalline compound (1)



(1)

comprising crystallizing compound (1) from crystallization solvent and controlling the amount of water in the crystallization solvent.

In another embodiment, a composition is provided that comprises crystalline free base compound (1) which is substantially free of the chloride salt of compound (1).

Crystalline compound (1) is useful in a method for therapy or prophylaxis of HCV infection comprising administering to a subject a therapeutic or prophylactic dose of crystalline compound (1). Another embodiment comprises the use of crystalline compound (1) for the manufacture of a medicament for the prevention or treatment of an HCV infection in a mammal (more specifically a human).

Another embodiment of this invention relates to pharmaceutical compositions of the crystalline formula (1) compound comprising at least one pharmaceutically acceptable excipient. In one embodiment the compound of formula (1) is formulated with an organic acid and optionally formulated into a

pharmaceutical dosage form such as a capsule. In another embodiment, crystalline compound (1) is micronized and formulated as a suspension.

Crystalline compound (1) or the pharmaceutical compositions of this invention are employed in the treatment or prophylaxis of hepatitis C.

Crystalline compound (1) exhibits improvements in pharmacological features and cost advantages, in particular improved purity, storage stability and manufacturing reproducibility. A particular advantage is its higher melting point as compared to the amorphous form.

Other features of this invention, including novel intermediates and product compositions, will be apparent from consideration of this application as a whole.

Figures

Figure 1 depicts an X-ray powder diffraction (XRPD) pattern obtained for crystalline compound (1) reference standard obtained by the method of example 1.

Figure 2 depicts another X-ray powder diffraction pattern obtained for crystalline compound (1).

Figure 3 is an X-ray powder diffraction pattern obtained for the amorphous form of compound (1) Research Lot 6, obtained by the method of Example 1a in WO 08/005519.

Figure 4 illustrates a DSC thermogram obtained for crystalline compound (1) reference standard, 1°C/min scan, obtained by the method of Example 1 below.

Figure 5 shows a DSC thermogram obtained for the amorphous form of compound (1) Research Lot 6, 5°C/min scan, obtained by the method of Example 1a in WO 08/005519.

Detailed Description of the Invention

Crystalline compound (1) is defined as a solid comprising compound (1) in which the constituent molecules are packed in a regularly ordered repeating pattern extending in all three spatial dimensions. Identification of crystallinity is readily accomplished in a number of ways known to those skilled in the art. Microscopic examination of the test composition often will reveal the presence of regular shapes, suggesting ordered internal structure. In the case of the crystal embodiment produced in example 1, the regular shape generally is rod or needle-like.

XRPD is another method for identifying crystalline compound (1). The regularly ordered structure of constituent molecules in a crystal diffracts

incident x-rays in a distinct pattern depicted as a spectrum of peaks. This pattern of peaks for crystalline compound (1) is shown in Figures 1 and 2. On the other hand, Figure 3 depicts an XRPD for substantially amorphous compound (1), which lacks distinct peaks. While the XRPD peaks for crystalline compound (1) may vary in intensity the same general pattern will be present in replicate x-ray diffraction analysis.

Crystalline compound (1) exhibits an XRPD dominant peak(s) at about 17 degrees theta 20, ordinarily 17.4 and 17.5. By "about" applicants mean within the typical variation in measurement of XRDP peaks. Such variations may result from the use of different instruments, instrument settings, batches of product, post-crystallization processing such as micronization or milling, and with varying sample preparation methods. In general, "about" means $\pm$ 0.5 degree theta 20. An example of this sort of variation can be seen by comparing Figures 1 and 2. In particular, peak intensity (e.g., at about 30) may vary due to crystal orientation effects.

Illustrative examples of other dominant peaks for crystalline compound (1) are at about 8, 10, 13, 16, 19 and 24 degrees theta 20, ordinarily 8.4, 10.0, 13.5, 15.7, 16.8, 16.9, 18.8 and 24.4. Any one or more of these peaks (but especially, 8, 10, 15.7, 16.7 and 16.9, with or without the peaks at about 17, are suitably employed to define the XRDP for crystalline compound (1).

The identification of a crystal form of compound (1) need not require the presence of any one or more of the dominant peaks seen in Figures 1 or 2. Rather, the presence or absence of dominant peaks ordinarily is taken into account with other diagnostic characteristics (e.g., DSC thermogram) to identify a candidate as crystalline compound (1).

Crystalline compound (1) also is characterized by DSC thermogram, which reveals an endothermic onset at about 235°C in differential scanning calorimetry profile. Typically, some variation in this measurement also will be encountered (usually, ± 1 - 3°C).

Crystalline compound (1) also is characterized by its heat of fusion (DH_f) of about 81 J/g (42 KJ/mole).

Crystalline compound (1) is made by a process comprising dissolving compound (1) in solvent and forming crystals therefrom. Typical solvents for use herein are ethyl acetate, isopropyl alcohol or a cosolvent containing ethyl acetate and isopropyl alcohol. Other suitable solvents are obtained from the solubility map in McConville, F.X. "Pilot Plant Real Book" (2002) which plots the dielectric constant and Hildebrand solubility parameter for a variety of solvents.

Solvents close to ethyl alcohol and isopropyl alcohol on the map (dielectric 2.5-20 and Hildebrand 15-24) are ethyl ether, isobutyl acetate, butyl acetate, anisole, chlorobenzene, chloroform, methyl acetate, THF, dichloromethane, dichloroethane, 1,2-dichlorobenzeke, methylisobutylketone, methylethylketone, cyclohexanone, acetone, 1-butanol, 2-methoxyethanol, isobutanol, 2-butanol, cyclohexanol, isoamyl alcohol, pyridine, methyl formate, 1-pentanol, and/or 2-butoxyethanol.

Some of these solvents would not be preferred due to toxicity issues, but this could be overcome by careful solvent removal from the product. It will be within the skill of the ordinary artisan to conduct laboratory screening to determine suitability of a candidate solvent for the preparation of crystalline

compound (1). Combinations of these solvents also fall within the scope of the invention.

A key finding facilitating the preparation of crystalline compound (1) is that the water content of the crystallization solvent must be controlled in order to obtain and/or optimize the production of crystalline product. For example, when using ethyl acetate as solvent, the upper limit on water content is about 0.6% to 0.9% by weight.

An additional consideration with regard to water content is its use to remove other forms of compound (1) that are less soluble than the crystalline free base in liquid lipophilic pharmaceutical carriers. For example the chloride salt of compound (1) is less soluble than the free base in the fatty acid solutions employed as carriers herein. In sufficiently large amounts such salts produce an undesirable haze in the pharmaceutical product. The final synthetic step of example 1 produces a mixture of free base together with minor amounts of the chloride salt. The haze-producing chloride salt is removed by first dissolving the product in a solvent containing a relatively high amount of water (about 3% - 10%) at alkaline pH. Refluxing in this solvent assures that there is enough water to back convert the chloride salt to the free base. Thereafter, the crystalline free base is crystallized from this solvent. This process optionally is repeated with decreasing water concentrations to gradually remove the chloride salt from the product. The final step is then accomplished with low water content (usually less than about 0.9% water) in order to crystallize the free base substantially free of the amorphous compound (1). In general, haze in the pharmaceutical preparation is not encountered when the chloride content in the final product is ordinarily less than about 100 ppm. The amounts of water employed will vary depending upon the concentration of contaminating

chloride salt and other experimental variables determinable by the skilled artisan. In summary, the water content of the crystallization solvent is controlled, both to convert chloride (or other relatively water soluble salts of compound (1)) and to avoid generation of amorphous compound (1).

The amount of permitted water for each function will vary depending upon the solvent or solvents employed for crystallization, the concentration of compound (1), the temperature of the crystallization step, the time of crystallization, the tolerable amount of amorphous compound (1), and other variables. Hence, it will be incumbent upon the artisan to determine the optimal water level for obtaining the desired results, usually by conducting a typical variable matrix study. The lowest water concentration for avoiding generation of the amorphous compound (1) is more a matter of practical economics. For example, 0.05% water by weight is acceptable.

In general, the final crystallization step is conducted in substantially anhydrous solvent. Substantially anhydrous solvent is defined as solvent containing a sufficiently small amount of water that the resulting product contains crystalline compound (1) and is substantially free of amorphous compound (1), typically less than about 40%, ordinarily less than about 30, 20, 10, 5, 3, 2 or 1% by weight of amorphous compound (1) in the total of all forms of compound (1) in the product composition.

In general, substantially anhydrous solvent will about 0.5% - 0.9% water by weight of the crystallization solvent. However, more water can be present if the desired product is permitted to contain the greater proportions of amorphous compound (1). However, it is optimal if the compound (1) composition is free of detectable amorphous compound (1).

The water content is controlled by any manner results in the proper amount of water in the crystallization step concerned. When formation of amorphous compound (1) is to be avoided, suitable techniques for minimizing or reducing the amount of water include adding drying agents and/or azeotropically removing water. It is most convenient to remove water during reflux dissolution of compound (1) just prior to crystallization. Of course, control of water content includes adding water as well, as will typically be the case during steps to convert the chloride salt.

Amorphous compound (1) optionally is used as starting material for crystallization (form conversion). Alternatively, crystallization is conducted directly from the final reaction products without an intermediate recovery of amorphous compound (1). The crystallization typically is conducted by providing or dissolving compound (1) in solvent or solvent mixture at reflux (sufficient to dissolve compound (1), about 1 to 5 hours), followed by cooling to about 18-23°C over 4 – 8 hours, then optionally agitated for about 8 to 20 hours at about 18-23°C. Agitation is optional but increases the rate of crystallization. Reflux is not critical since all that is necessary is that compound (1) be placed in solution. However, refluxing compound (1) has the advantage of rapidly dissolving compound (1) and azeotropically removing water at the same time. Water is controlled before crystallization starts or during crystallization, or both, although in general it is best to reduce water below the desired limit before any compound (1) can precipitate as the amorphous polymorph.

Generation of amorphous material is optimized by using relatively longer crystallization times, higher temperatures and lower concentrations of compound (1). Determining the various optimal crystallization process parameters are well within the skill of the ordinary artisan.

An embodiment herein is a composition made by the process of combining crystalline compound (1) with a pharmaceutically acceptable excipient and forming a pharmaceutical dosage form such as a tablet or capsule. The resulting product need not contain crystalline compound (1). While it is expected that dosage forms made from crystalline compound (1) will contain only compound (1) in crystalline form. However, in some embodiments the crystalline compound (1) is an intermediate for dissolution in the carrier or excipient.

The crystalline compound (1) of this invention is administered to a subject mammal (including a human) by any means well known in the art, i.e. orally, intranasally, subcutaneously, intramuscularly, intradermally, intravenously, intra-arterially, parenterally or by catheterization in a therapeutically effective amount, i.e., an HCV-inhibiting amount or an HCV-replication inhibiting amount. This amount is believed to be an amount that ensures a plasma level of about 100 nM, 3 times the protein-adjusted EC90. This ordinarily is expected to be achieved by daily oral administration of about 0.5 – about 5 mg/kg, typically about 0.7 to 2.2 mg/kg, most ordinarily about 1.2 mg/kg bodyweight for humans.

The optimal dosage of the compound of this invention will depend upon many factors known to the artisan, including bioavailability of the compound in a given formulation, the metabolism and distribution of the compound in the subject, the fasted or fed state of the subject, selection of carriers and excipients in the formulation, and other factors. Proper dosing typically is determined in the preclinical and clinical settings, and is well within the skill of the ordinary artisan. The therapeutically effective amount of the compound of this invention optionally is divided into several sub-units per day or is administered daily or

in more than one day intervals, depending upon the nature of the infection, the patient's general condition and the formulation of the compound of this invention. Generally, the compound is administered twice daily.

The compound of this invention is employed in concert with other agents effective against HCV infections. They optionally are administered separately in a course of therapy, or are combined with compound (1) in a unitary dosage form such as tablet, iv solution or capsule. Such other agents include, for instance, interferon-alpha, ribavirin, and/or compounds falling within the disclosures of EP1162196, WO 03/010141, WO 03/007945, WO 00/204425 and/or WO 03/010140 (and other filings within their patent families). Other agents for administration in a course of therapy with the compound of this invention include compounds now in clinical trials, in particular HCV protease inhibitors such as VX-950 (Vertex Pharmaceuticals), SCH 5030347 (Schering Plough) and BILN-2061 (Boehringer Ingelheim), nucleoside HCV inhibitors such as NM283, NM107 (both Idenix/Novartis) and R1626 (Hoffmann-LaRoche), and non-nucleoside HCV inhibitors including HCV-086 and -796 (both ViroPharma/Wyeth). Supplementary antiviral agents are used in conventional amounts. If the efficacy of the compound of this invention and the supplementary compound are additive then the amounts of each active agent optionally are commensurately reduced, and more so if the agents act synergistically. In general, however, the agents are used in their ordinary active amounts in unitary combination compositions.

Co-administered agents generally are formulated into unitary compositions with the compound of this invention so long as they are chemically compatible and are intended to be administered by the same route.

If not, then they optionally are provided in the form of a medical kit or package containing the two agents in separate repositories or compartments.

The compound of this invention typically is provided as the free base, but also optionally is prepared as a salt. Salts typically are prepared by acid addition of organic and/or inorganic acids to the free base. Examples include (1) inorganic acids such as hydrohalogen acids, e.g. hydrochloric or hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and sulfamic acids; or (2) organic acids such as acetic, propanoic, hydroxyacetic, benzoic, 2-hydroxypropanoic, 2-oxopropanoic, lactic, fumaric, tartaric, pyruvic, maleic, malonic, malic, salicylic (e.g. 2-hydroxybenzoic), p-aminosalicylic, isethionic, lactobionic, succinic, oxalic and citric acids; organic sulfonic acids, such as methanesulfonic, ethanesulfonic, benzenesulfonic, p-toluenesulfonic, C1-C6 alkylsulfonic, benzenesulfonic, p-toluenesulfonic, and cyclohexanesulfamic acids. Typical salts are the chloride, sulfate, bisulfate, mesylate, besylate, esylate, phosphate, oxalate, maleate, succinate, citrate, malonate, and/or fumarate salts. Also included within the scope of this invention are the salts of the compound of this invention with one or more amino acids, typically naturally-occurring amino acids such as one of the amino acids found in proteins. The acidic counterion desirably is physiologically innocuous and non-toxic or otherwise pharmaceutically acceptable, unless the salt is being used as an intermediate in preparation of the compounds whereupon toxicity is not relevant. Ordinarily, compound (1) will be administered as the free base, but suitable salts include mesylate (methanesulfonic acid) and HCl.

The compound of this invention includes the solvates formed with the compound of this invention or their salts, such as for example hydrates, alcoholates and the like.

The pharmaceutical compound of this invention optionally is formulated with conventional pharmaceutical carriers and excipients, which will be selected in accord with ordinary practice. Tablets will contain excipients, glidants, fillers, binders and the like. Aqueous formulations are prepared in sterile form, and when intended for delivery by other than oral administration generally will be isotonic. Formulations optionally contain excipients such as those set forth in the "Handbook of Pharmaceutical Excipients" (2005) and include ascorbic acid and other antioxidants, chelating agents such as EDTA, carbohydrates such as dextrin, hydroxyalkylcellulose, hydroxyalkylmethylcellulose and/or organic acids such as oleic acid or stearic acid.

The term "pharmaceutically acceptable carrier" as used herein means any material or substance formulated with the active ingredient in order to facilitate its preparation and/or its application or dissemination to the site to be treated. Suitable pharmaceutical carriers for use in the compositions of this invention are well known to those skilled in the art. They include additives such as wetting agents, dispersing agents, adhesives, emulsifying agents, solvents, glidants, coatings, antibacterial and antifungal agents (for example phenol, sorbic acid, chlorobutanol), and isotonic agents (such as sugars or sodium chloride), provided that the same are consistent with pharmaceutical practice, i.e. they are not toxic to mammals.

The pharmaceutical compositions of the present invention are prepared in any known manner, for instance by homogeneously mixing, coating and/or grinding the active ingredients in a one-step or multi-step procedure, with the selected carrier material and, where appropriate, other additives such as surface-active agents. Compositions containing the compound of this invention

formulated into microspheres (usually having a diameter of about 1 to 10 gm) are useful as controlled or sustained release formulations.

In one optional formulation, compound (1) is comminuted to a finely divided form, typically to an average particle size at any point within the range of about 1 - 20 microns. The product of example 1 is rods or needles and exhibits a range of crystal length, typically about 25 – 40 microns. These optionally are micronized in a Jet mill-00 at about 60-80 psi to obtain particles of about 3-4 microns and having surface area of about 7-8 square meters/g. However, the starting crystal sizes will vary from lot to lot and the degree of micronization is a matter of choice. Accordingly, micronized crystalline compound (1) is simply defined as crystal or amorphous compound (1) that has been subject to a micronization process such as the exemplary one described here. Neither the size nor surface area of the resulting particles is critical. The micronized compound (1) is suspended in aqueous solution, optionally aided by a suspending agent, emulsifiers and/or surfactant as further described below.

Typically, the pharmaceutical formulation is a solubilized form of compound (1) where crystalline compound (1) is dissolved in an appropriate solvent or solubilizing agent, or combinations thereof. Crystalline compound (1) is solubilized in a pharmaceutically acceptable excipient for administration therapeutically or prophylactically.

Suitable solutions of compound (1) for pharmaceutical preparations include water together with various organic acids (typically C4 – C24) usually fatty acids like capric, oleic, lauric, capric, palmitic and/or myristic acid. The fatty acids are optionally saturated or unsaturated, or mixtures thereof. In addition, polyethylene glycols (PEGs) and/or short, medium, or long chain

mono, di, or triglycerides are employed supplementary to, or in place of, the organic acids. Pegylated short, medium or long chain fatty acids optionally also are used in the same fashion.

The most common organic acids are the carboxylic acids whose acidity is associated with the carboxyl group -COOH. Sulfonic acids, containing the group OSO_3H, are relatively stronger acids for use herein. In general, the acid desirably contains a lipophilic domain. Mono- or di-carboxylic acids are suitable.

Suitable surface-active agents optionally are used with any of the formulations of this invention (any one or more of the following agents, typically any one of them). Such agents also are known as emulgents or emulsifiers, and are useful in the pharmaceutical compositions of the present invention. They are non-ionic, cationic and/or anionic materials having suitable emulsifying, dispersing and/or wetting properties. Suitable anionic surfactants include both water-soluble soaps and water-soluble synthetic surface-active agents. Suitable soaps are alkaline or alkaline-earth metal salts, unsubstituted or substituted ammonium salts of higher fatty acids (C_{10}-C_{22}), e.g. the sodium or potassium salts of oleic or stearic acid, or of natural fatty acid mixtures obtainable from coconut oil or tallow oil. Synthetic surfactants include sodium or calcium salts of polyacrylic acids; fatty sulphonates and sulphates; sulphonated benzimidazole derivatives and alkylarylsulphonates. Fatty sulphonates or sulphates are usually in the form of alkaline or alkaline-earth metal salts, unsubstituted ammonium salts or ammonium salts substituted with an alkyl or acyl radical having from 8 to 22 carbon atoms, e.g. the sodium or calcium salt of lignosulphonic acid or dodecylsulphonic acid or a mixture of fatty alcohol sulphates obtained from natural fatty acids, alkaline or alkaline-

earth metal salts of sulphuric or sulphonic acid esters (such as sodium lauryl sulphate) and sulphonic acids of fatty alcohol/ethylene oxide adducts. Suitable sulphonated benzimidazole derivatives preferably contain 8 to 22 carbon atoms. Examples of alkylarylsulphonates are the sodium, calcium or alcoholamine salts of dodecylbenzene sulphonic acid or dibutyl-naphthalenesulphonic acid or a naphthalene-sulphonic acid/formaldehyde condensation product. Also suitable are the corresponding phosphates, e.g. salts of phosphoric acid ester and an adduct of p-nonylphenol with ethylene and/or propylene oxide, or phospholipids. Suitable phospholipids for this purpose are the natural (originating from animal or plant cells) or synthetic phospholipids of the cephalin or lecithin type such as e.g. phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerine, lysolecithin, cardiolipin, dioctanylphosphatidyl-choline, dipalmitoylphoshatidyl -choline and their mixtures. Aqueous emulsions with such agents are within the scope of this invention.

Suitable non-ionic surfactants include polyethoxylated and polypropoxylated derivatives of alkylphenols, fatty alcohols, fatty acids, aliphatic amines or amides containing at least 12 carbon atoms in the molecule, alkylarenesulphonates and dialkylsulphosuccinates, such as polyglycol ether derivatives of aliphatic and cycloaliphatic alcohols, saturated and unsaturated fatty acids and alkylphenols, said derivatives preferably containing 3 to 10 glycol ether groups and 8 to 20 carbon atoms in the (aliphatic) hydrocarbon moiety and 6 to 18 carbon atoms in the alkyl moiety of the alkylphenol. Further suitable non-ionic surfactants are water-soluble adducts of polyethylene oxide with poylypropylene glycol, ethylenediaminopolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain, which adducts contain 20 to 250 ethyleneglycol ether groups and/or 10 to 100 propyleneglycol ether groups.

Such compounds usually contain from I to 5 ethyleneglycol units per propyleneglycol unit. Representative examples of non-ionic surfactants are nonylphenol -polyethoxyethanol, castor oil polyglycolic ethers, polypropylene/polyethylene oxide adducts, tributylphenoxypolyethoxyethanol, polyethyleneglycol and octylphenoxypolyethoxyethanol. Fatty acid esters of polyethylene sorbitan (such as polyoxyethylene sorbitan trioleate), glycerol, sorbitan, sucrose and pentaerythritol are also suitable non-ionic surfactants.

Suitable cationic surfactants include quaternary ammonium salts, particularly halides, having 4 hydrocarbon radicals optionally substituted with halo, phenyl, substituted phenyl or hydroxy; for instance quaternary ammonium salts containing as N-substituent at least one C8 - C22 alkyl radical (e.g. cetyl, lauryl, palmityl, myristyl and oleyl) and, as further substituents, unsubstituted or halogenated lower alkyl, benzyl and/or hydroxy-lower alkyl radicals.

A more detailed description of surface-active agents suitable for this purpose is found in "McCutcheon's Detergents and Emulsifiers Annual" (MC Publishing Crop., Ridgewood, New Jersey, 1981), "Tensid-Taschenbucw", 2nd ed. (Hanser Verlag, Vienna, 1981) and "Encyclopedia of Surfactants," (Chemical Publishing Co., New York, 1981).

The compound of this invention is administered by any route appropriate to the condition to be treated, such as oral, rectal, nasal, topical (including ocular, buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural). The preferred route of administration may vary with for example the condition of the recipient, but is generally oral.

Formulations of the compound of this invention for oral administration usually are presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granular form; as a solution or suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The compound of this invention optionally is presented as a bolus, electuary or paste.

A tablet is made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets are prepared by compressing in a suitable machine the compound of the invention in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active and/or dispersing agent. Molded tablets typically are made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

The formulations are optionally applied as a topical ointment or cream containing the active ingredient(s) in an amount of, for example, 0.075 to 20% w/w (including active ingredient(s) in a range between 0.1% and 20% in increments of 0.1% w/w such as 0.6% w/w, 0.7% w/w, etc), preferably 0.2 to 15% w/w and most preferably 0.5 to 10% w/w. When formulated in an ointment, the compound is employed with a paraffinic or a water-miscible ointment base. Alternatively, the compound is formulated in a cream with an oil-in-water cream base. If desired, the aqueous phase of the cream base may include, for example, at least 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or

more hydroxyl groups such as propylene glycol, butane 1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol (including PEG400) and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active ingredient through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide and related analogs.

The oily phase of the emulsions of this invention is constituted from known ingredients in a known manner. While this phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Optionally, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties. Thus the cream should optionally be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties

required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active ingredient. The active ingredient is optionally present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% particularly about 1.5% w/w.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate. Formulations suitable for nasal administration wherein the carrier is a solid include a coarse powder having a particle size for example in the range 20 to 500 microns (including particle sizes in a range between 20 and 500 microns in increments of 5 microns such as 30 microns, 35 microns, etc), which is administered by aerosol or powder inhalers, of which numerous examples are available. Suitable formulations wherein the carrier is a liquid, for administration as for example a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations

containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations are presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

The compound of this invention optionally is formulated into controlled release compositions in which the release of the compound is controlled and regulated to allow less frequency dosing or to improve the pharmacokinetic or toxicity profile of the invention compound. Controlled release compositions are prepared in accord with known methods, many of which involve formulating the active compound with one or more polymer carriers such a polyester, polyamino acid, polyvinyl pyrrolidone, ethylene-vinyl acetate copolymer, methylcellulose, carboxymethylcellulose and/or protamine sulfate. The rate of drug release and duration of action optionally is controlled by incorporating the active ingredient into particles, e.g. microcapsules, of a polymeric substance such as hydrogels, polylactic acid, hydroxymethylcellulose, polymethyl methacrylate and the other above-described polymers. Also suitable are colloid drug delivery systems such as liposomes, microspheres, microemulsions,

nanoparticles, nanocapsules and so on. Depending on the route of administration, the pharmaceutical composition, e.g., tablets, may require protective coatings.

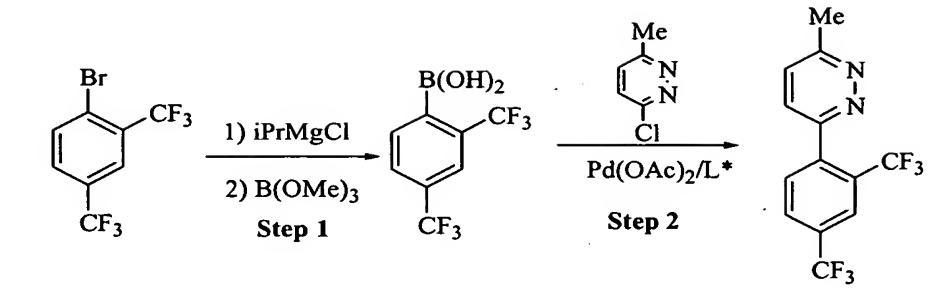
The invention will be more fully appreciated by reference to the following examples, which are to be considered merely illustrative and not limiting the scope of the invention.

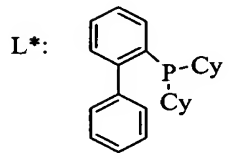
Composition percentages are by weight unless otherwise apparent from the context.

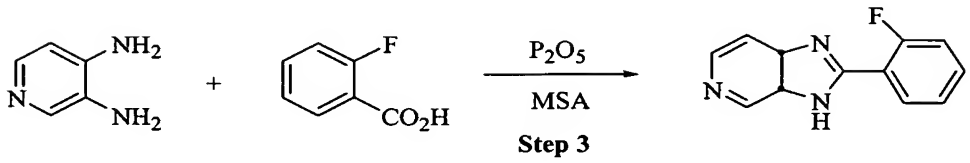
Example 1

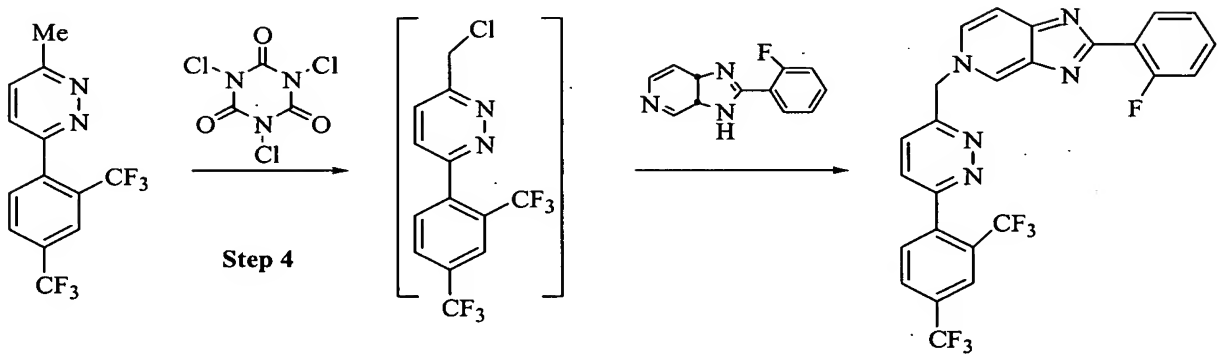
Synthesis of Crystalline 5-((6-(2,4-Bis(trifluoromethyl)phenyl]pyridazin-3-yl)methyl)-2-(2-fluorophenyl)-5*H*-imidazo[4,5-*c*]pyridine

Scheme 1

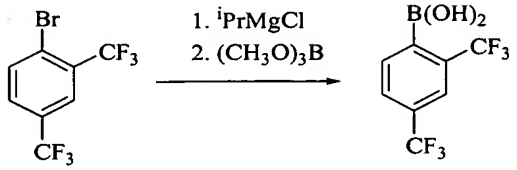








Step 1



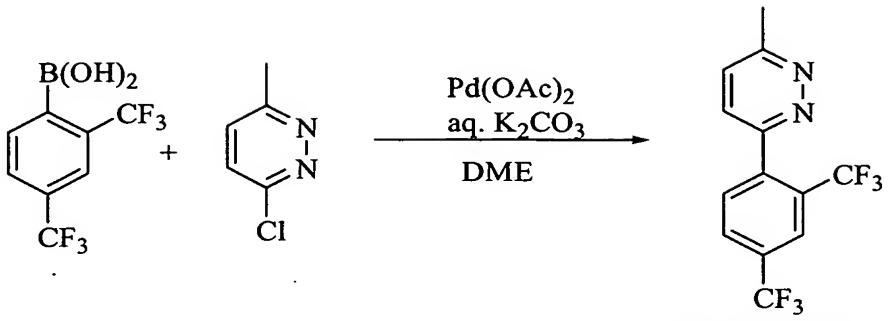
To a reactor, containing 2,4-bis(trifluoromethyl)bromobenzene (1.00 eq) and tetrahydrofuran (THF), was charged Isopropyl magnesium chloride

(^{i}PrMgCl) (2 M in THF, 1.14 eq) while maintaining the content at -10°C. The mixture was agitated at -10°C until the reaction was completed by HPLC analysis. The resultant mixture was transferred to the second reactor, containing trimethyl borate (2.26 eq) and THF held at a temperature of -10°C. The reaction was then monitored by HPLC until 1,3-bis(trifluoromethyl)benzene was not more than 2%. Aq. HCl (aqueous hydrochloric acid), prepared from water and concentrated 37% hydrochloric acid (HCl) were then added to quench the reaction while maintaining the content at not more than 25°C. After agitating the content for 1-2 h and settling for ca. 30 minutes, the layers were separated. The organic layer was washed with brine solution mixed with water and then concentrated under vacuum. Heptane was charged and the content was further concentrated under vacuum. The operations were repeated one more time. Heptane was then charged and the resultant slurry is cooled to 3°C, and agitated at the temperature for 4-6 h.

The product was filtered, washed with heptane twice and dried under vacuum at a maximum of 40°C.

Material	M.W.	v/w Ratio	w/w Ratio	Mole Ratio
2,4-Bis(trifluoromethyl)-bromobenzene	293.00	—	1.00	1.00
Heptanes	100.21	13.20	9.00	—
Hydrochloric acid, concentrated (37%)	36.50	0.42	0.50	—
Isopropyl magnesium chloride (2 M in Tetrahydrofuran)	102.85	1.95	1.90	1.14
Sodium Chloride (NaCl)	58.11	—	0.60	—
Tetrahydrofuran (THF)	72.11	4.50	4.00	—
Trimethyl borate	103.91	0.86	0.80	2.26
Water	18.02	8.90	8.90	—

Step 2



Compound 2a

3-Chloro-6-methylpyridazine (1.00 eq), 2-(dicyclohexylphosphino)biphenyl (0.05 eq), 2,4-bis(trifluoromethyl)phenylboronic acid (1.85 eq), 1,2-dimethoxyethane and aqueous potassium carbonate solution were all charged into a reactor. After degassing three times with nitrogen, palladium acetate (0.025 eq) was charged and the content is heated and agitated under reflux until the reaction was deemed complete.

The reaction mixture was cooled to 22°C. Heptane was charged, followed by addition of Celite. After agitating for ca. 30 minutes at 22°C, the mixture was filtered into the first reactor, rinsing forward with a mixture of 1,2-dimethoxyethane and Heptanes. The layers of the filtrate are separated.

To the organic layer was charged borane trimethylamine complex (0.03 eq), water, and acetic acid. The resultant mixture with a pH at maximum 4 was agitated for 1-2 h at 22°C and then refluxed at ca. 80°C for 2-3 h. After cooling back to 22°C, the mixture was adjusted to pH 10-11 with addition of 5% aq. sodium hydroxide while maintaining the content at 22°C and then agitated for 1-2 h. The mixture was filtered and the layers were separated. The aq. layer was disposed of and the organic layer was filtered through ZetaCarbon cartridges

into the in-process cleaned first reactor, rinsing forward with 1,2-dimethoxyethane through the carbon cartridges.

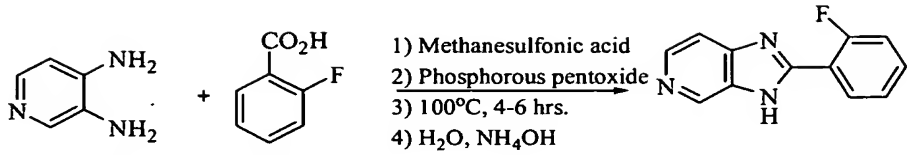
The filtrate was concentrated under vacuum with a maximum jacket setting of 60°C. Heptane was charged and the contents were further concentrated under vacuum with a maximum jacket setting of 60°C. Additional Heptane was charged to the concentrate and the 1,2-dimethoxyethane (DME) content (maximum 0.5%) of the mixture was checked by NMR. After adjusting to 85°C and agitating for ca. 1 h, the mixture was polished filtered hot through a filter into the second reactor.

The filtrate in the second reactor was adjusted to reflux and then agitated for 1 h. With ramp cooling and moderate agitation, the mixture is cooled from reflux to 0 to 6°C over a period of minimum 4 h and then agitated at 0 to 6°C for 1 h.

The product was filtered, washed with ambient temperature Heptanes and dried under vacuum at a maximum of 40°C until loss on drying is maximum 1%.

Materials	M.W.	w/w Ratio	Mole Ratio	v/w Ratio
2,4-Bis(trifluoromethyl)phenyl-boronic acid	257.92	4.00	1.85	—
Borane trimethylamine complex	72.92	0.018	0.03	—
3-Chloro-6-methylpyridazine	128.56	1.00	1.00	—
Diatomaceous earth (celite)	N/A	0.30	—	—
Di(cyclohexyl)phosphinobiphenyl	350.49	0.14	0.05	—
1,2-Dimethoxyethane	90.12	12.00	—	13.80
Drinking water	18.02	3.75	—	3.75
Glacial acetic acid	60.05	0.05	0.10	—
Heptanes	100.21	20.40	—	29.80
Palladium (II) acetate	224.49	0.044	0.025	—
Potassium carbonate,	138.21	2.15	2.00	—
Sodium hydroxide, 5% solution	40.00	—	—	—

Step 3



3,4-Diaminopyridine MW = 109.13 | 2-Fluorobenzoic Acid MW = 140.11 | Core (2) MW = 213.2

To a reactor was charged methanesulfonic acid, followed by phosphorus pentoxide (1.00 eq) in portions while maintaining the content at 23°C. 3,4-Diaminopyridine (1.00 eq) was charged in portions while maintaining the content at 20 to a maximum of 50°C. 2-Fluorobenzoic acid (1.09 eq) was then charged. The mixture was heated to 100°C and the reaction was monitored by HPLC until completion.

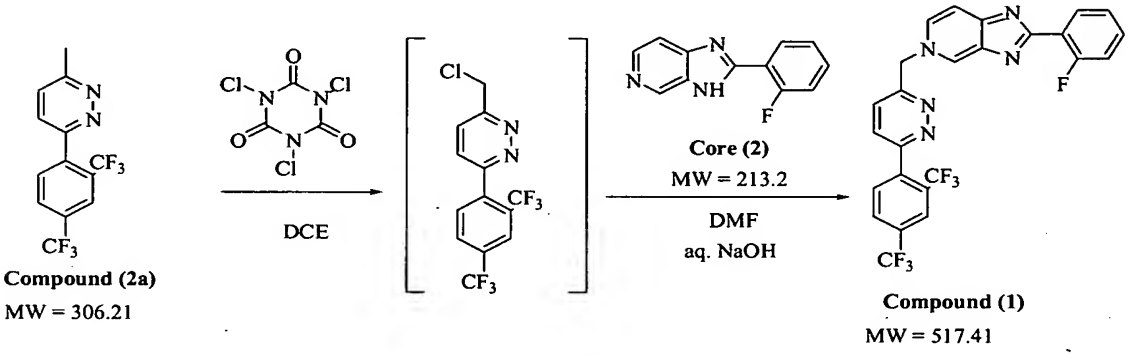
The content was adjusted to 10°C and water was charged while maintaining the content at a maximum of 25°C. After agitating the mixture at this temperature for 1 h, it was filtered into a second reactor.

To the filtrate in the second reactor was charged 27% ammonium hydroxide until the pH was in between 6.0-6.5. The content temperature was kept at a maximum of 30°C. The resultant thin slurry was agitated at 22°C for a minimum of 1 h and 27 % ammonium hydroxide was further charged, until the pH was between 8.0-9.3. The slurry was further agitated at 22°C for a minimum of 2 h.

The product was filtered, washed with water twice, and dried at a maximum of 60°C under vacuum, until the water content is not more than 1%. If necessary, the product is milled to remove large lumps.

Materials	M.W.	w/w Ratio	Mole Ratio	v/w Ratio
Ammonium hydroxide, 27%	35.05	—	—	—
3,4-Diaminopyridine	109.13	1.00	1.00	—
Drinking water	18.02	24.00	—	24.00
2-Fluorobenzoic acid	140.11	1.40	1.09	—
Methanesulfonic acid	96.10	7.00	—	4.70
Phosphorous pentoxide	141.94	1.30	1.00	—

Step 4



To a reactor is charged compound 2a (1.24 eq), methylene chloride and trichloroisocyanuric acid (0.491 eq). The mixture wxas adjusted to reflux and agitated under reflux until the reaction is complete.

The reaction mixture was cooled to 22°C and celite was charged. After agitating for minimum of 30 minutes, the mixture was filtered into and rinsed forward with methylene chloride 3 times into the second reactor. The filter cake was disposed of. To the filtrate in the second reactor was charged 3% aq. sodium hydroxide whilst maintaining the contents at 22°C. The mixture was agitated for 1-2 h and the layers were separated. The bottom organic layer was transferred to the in-process cleaned first reactor and concentrated under vacuum with a maximum jacket temperature of 45°C. Methylene chloride was

charged and the mixture was polish filtered to the in-process cleaned second reactor.

The filtrate was concentrated under vacuum with a maximum jacket temperature of 45°C. Dimethylformamide (DMF) was charged and the contents are further concentrated. The mixture was adjusted to 22°C and DMF was charged, followed by compound core 2 (1.00 eq) and 10% aq. sodium hydroxide while maintaining the content at 22°C. The resultant mixture was agitated at 22°C until the reaction was monitored by HPLC analysis. Over the reaction period, the pH of the content was monitored and 10% aq. sodium hydroxide was added as required to maintain the pH at 11-12 by pH meter. After the reaction, 10% aq. sodium hydroxide was charged while maintaining the contents at 22°C. The mixture was diluted with DMF and agitated for 2 h. The mixture was filtered over a minimum of 1 h into the first in-process cleaned first reactor, containing water, whilst maintaining the contents at 16°C and then rinsing forward with DMF. The resultant slurry was agitated for 1-3 h at 22°C.

The crude product was filtered and washed with water and then methyl tertiary butyl ether (MTBE). The wet crude product was discharged from the filter and transferred into the first reactor; and ethyl acetate (EtOAc) was charged. The mixture was heated to reflux and agitated at reflux temperature until all the solids are dissolved. The water level must be less than 6.0%. With ramp cooling, the content was adjusted to 22°C over a minimum of 4 h.

The crystallized product was filtered and washed with EtOAc and then charged back to the first reactor. Ethyl acetate (EtOAc) was added. The mixture was heated to reflux and agitated at the temperature until all the solids are dissolved. The water level must be not less than 1.0%. The mixture was filtered,

hot, through a polishing filter into the second reactor (EtOAC preconditioned), rinsed forward with EtOAc.

The product was concentrated under atmospheric pressure. After adjusting to 65°C and charging in EtOAc, the pot was adjusted to reflux and agitated at reflux for ca. 30 minutes. Water content was checked and if the water level was more than 0.2%, the same cycle was repeated.

Once the water level was at maximum 0.2%, the content was adjusted to reflux and then agitated under reflux for 1-3 h. With ramp cooling, the content was adjusted to 22°C over minimum 4 h and then agitated at the temperature for minimum of 8 h.

The product was filtered, washed with EtOAc and dried under vacuum at maximum of 60°C. The product was then milled.

Materials	**M.W.**	**w/w Ratio**	**Mole Ratio**	**v/w Ratio**
3-(2,4-Bis(trifluoromethyl)-phenyl)-6-methylpyridazine,	306.21	1.00	1.00	—
t-Butyl methyl ether	88.15	—	—	—
Diatomaceous Earth (Celite)			—	—
N,N-Dimethylformamide	73.10	6.90	—	7.30
Drinking water	18.02	27.72	—	27.72
Ethyl acetate	88.11	33.90	—	37.70
2-(2-Fluorophenyl)-imidazo-[4,5-c]pyridine, GS-9133	213.21	0.560	0.78	—
Methylene chloride	84.93	16.50	—	12.50
Sodium hydroxide	40.00	0.276	—	—
Trichloroisocyanuric acid	232.41	0.315	0.415	—

5

Nuclear Magnetic Resonance (^{1}H-, ^{13}C-, and ^{19}F-NMR) Spectra

Nuclear magnetic resonance (NMR) spectra of compound (1) is consistent with the proposed structure. The ^{13}C, ^{19}F, and ^{1}H-NMR spectra of compound (1) in DMSO-d_6 were measured using a Varian UnityInova-400 FT-
10 NMR spectrometer. Spectra are shown in the table below. The NMR chemical shift assignments were established using 2D correlation experiments (COSY, HSQC, HMBC and HSQCTOCSY).

^{1}H- and ^{13}C-NMR chemical shift assignments for Compound (1) reference standard

Atom	δC/ppm (DMSO-d_6)	δF/ppm (DMSO-d_6)	δH/ppm (DMSO-d_6)
1A	140.16		
2A	128.32 (q[a], J_{CF} = 32 Hz)		
3A	123.61, m		8.24 (m, 1 H)
4A	130.27 (q, J_{CF} = 34 Hz)		
5A	129.54 (q, J_{CF} = 3 Hz)		8.22 (m, 1 H)
6A	133.36		7.88 (m, 1 H)
7A	123.20 (q, J_{CF} = 273 Hz)	–56.4[b]	
8A	123.02 (q, J_{CF} = 275 Hz)	–62.0[b]	
1	158.76		
2B	128.16		8.01 (d, 1 H, J = 8.4 Hz)
3B	126.20		7.95 (d, 1 H, J = 8.8 Hz)
4B	157.70		
5B	60.49		6.17 (s, 2 H)
2C	131.86		8.31 (m, 1 H)
3C	112.63		7.86 (m, 1 H)
4C	155.44		
6C	168.11 (d, J_{CF} = 6 Hz)		
8C	145.08		
9C	133.06		9.25 (s, 1 H)
1D	123.11 (d, J_{CF} = 10 Hz)		
2D	160.46 (d, J_{CF} = 254 Hz)	–111.7	
3D	116.59 (d, J_{CF} = 22 Hz)		7.29 (m, 1 H)
4D	130.84 (d, J_{CF} = 8 Hz)		7.46 (m, 1 H)
5D	124.13 (d, J_{CF} = 4 Hz)		7.31 (m, 1 H)
6D	131.72 (d, J_{CF} = 2 Hz)		8.35 (m, 1 H)

a. multiplicity, s: singlet, d: doublet, q: quartet, m: multiplet
b. interchangeable signals

Differential Scanning Calorimetry

Compound (1) samples (amorphous) designated "Research lot 6" were made according to the method published as Example 1a in WO 08/005519, which is hereby incorporated by reference in its entirety. The remaining samples were crystalline compound (1). The samples were subjected to measurement using a Differential Scanning Calorimetry (DSC) apparatus (DSC2010, manufactured by TA Instruments Corporation), under nitrogen atmosphere, sample weight 5 ±1 mg, temperature rise rate: either 1°C per min, 5°C per min or 10°C per min, open aluminum pan, and indium standard as a reference. The enthalpy, extrapolated onset temperature and apex temperature at an endothermic peak on the obtained DSC curve were determined.

The DSC results for Research lot 6 and representative crystalline free base compound (1) batches are summarized in Table 1 and Figures 4 and 5, respectively. When the crystal form of compound (1) was subjected to DSC scan at 1°C/min, the enthalpy of the endothermic peak is about 80 J/g, and the extrapolated onset temperature is 233.2°C ±2.0°C. The apex of the endothermic peak is 233.9°C ±3.0°C.

Table 1. Example DSC values obtained for Compound (1) batches

	10 °C/min scan		1 °C/min scan		
	peak onset	main peak	peak onset	main peak	Enthalpy (J/g)
9190 Ref Std	235.8	237.2	233.7	234.6	89.5
9190-A-1	n/a	n/a	234.8	234.0	--
9190-B-1 Crop 1	235.2	237.4	231.6	232.2	78.5
9190-B-1 Crop 2	236.1	238.5	234.3	235.6	80.9
**Research Lot 6	220.2	221.3	pending	pending	39.1

Note: All °C excecpt for enthalpy
**5 °C/min scan reported for Lot 6

X-Ray Powder Diffractometry – Study 1

Samples made by example 1a of WO 05/063744 and by the method of this invention were analyzed in the as received condition, only mixing with a spatula prior to analysis. A sample was fixed to an aluminum cell, and the measurement was performed using an X-ray powder diffractometer (XRD-6000, Shimadzu Lab X, manufactured by Shimadzu Corporation, X-ray source: Cu—Kα1 ray, tube voltage: 35 kV, tube electric current: 40 mA, scan speed: 2º per min, continuous scan mode, sampling pitch: 0.02º, scan range: 4 – 35º, β axis rotation: 60 rpm).

Non-micronized, ascicular compound (1) crystals obtained by the method of this invention have an X-ray powder diffraction pattern having characteristic diffraction peaks at diffraction angles 2θ (º) of 13.46, 15.59, 16.90, 17.48, 23.05 and 30.15 as measured by X-ray powder diffractometer (Figure 1). Note that the non-micronized "high melt" 235°C melt ascicular crystal form of compound (1) tested in this example shows some effects due to preferred

orientation and particle size. As a result, Figure 1 should be considered merely exemplary because varying the crystal size and orientation will change the magnitude of the peaks in the plot. Additionally, the diffraction peak value at the above mentioned diffraction angle 2θ (º) may show slight measurement error due to the measurement instrument or measurement conditions and the like. Typically, the measurement error generally is within the range of about ±0.3. The specification for the Shimadzu XRD-6000 is ±0.04. Further, some variation in peak positions can be expected due to product and experimental variation, so they must be considered approximate.

The 220°C "low melt" solid state form of compound (1) comprised by product made according to the example 1a method (or in the method herein prior to the reslurry step) gives an X-ray powder diffraction pattern consistent with amorphous material (Figure 3).

Compound (1) by the method of this invention typically exhibits intrinsic solubility of 0.7 micrograms/ml, a pKa of 5.8, log P of 2.8; and geometric mean (3 lots) pH solubility profile at pH 2 of 458 micrograms/ml and at pH 7.3, 0.7 micrograms/ml. Geometric mean solubility (3 lots) in simulated intestinal fluids (fasted: pH 6.4, 0.75 mM lecithin, 3 mM sodium taurocholate, 270 mOsmol; fed: pH 5.0, 3.75 mM lecithin, 15 mM sodium taurocholate, 635 mOsmol) were 19.1 micrograms/ml (fasted) and 122 micrograms/ml (fed).

Measured parameters vary from lot to lot, so all of the foregoing parameters except molecular weight should be considered to be approximate.

Titration with acids revealed higher solubility with mesylate (>20 mg/ml) compared to the chloride (about 0.6 mg/mL) or sulfate (about 0.5 mg/mL) counterions.

X-Ray Powder Diffractometry – Study 2

Another sample of crystalline compound (1) prepared by the method of this invention was analyzed in the same fashion as Study 1 except that the X-ray powder diffractometer was a PANalytical X'Pert Pro MPD PW3040 Pro, manufactured by PANalytical Inc., using X-ray source: Cu—Kα ray (1.54059 Å), tube voltage: 45 kV, amperage: 40 mA, scan range: 1 – 55 º2θ, step size: 0.008 º2θ, collection time: 3373 s, scan speed: 0.9º per min, slit: DS: 1/2º, SS: 1/4º, revolution time: 0.5 s, mode: transmission. The results are depicted in Figure 2.

Example 2

Formulation of Compositions Using Compound (1)

Crystalline compound (1) is used as an intermediate to produce pharmaceutically acceptable solutions. The following examples are made on a weight by weight basis to achieve 10% w/w active. To make 12 kg solution, exemplary quantitative compositions of compound (1) capsules, 20 mg and 40 mg are listed below.

Quantitative composition of Compound (1) capsules, 20 mg and 40 mg

Components	% w/w	Capsule Unit Formula (mg/unit)		Compendial Reference	Function
		20 mg	40 mg		
Compound 1	10.00	20.0	40.0	None	Active ingredient
Oleic Acid	84.55	169.1	338.2	NF	Solvent
Polysorbate 80	5.00	10.0	20.0	NF	Surfactant
Butylated Hydroxytoluene (BHT)	0.10	0.2	0.4	NF	Antioxidant
Butylated Hydroxyanisole (BHA)	0.35	0.7	1.4	NF	Antioxidant
Capsule Sealing Solution[a]					Capsule sealant
Ethanol	---[b]	---[b]	---[b]	USP	---
Purified water	---[b]	---[b]	---[b]	USP	---
Capsule Shell, Size 0 Licaps™ White Opaque	N/A	1 each	1 each	None	Capsule shell
Total	**100.00**	**200.0**	**400.0**		

[a] Composition is 1:1 w/w ethanol:water solution.

[b] Removed during the capsule sealing process.

- Container/vessel: 12kg stainless steel
- Weigh the following in order:
- 0.012 kg butylated hydroxytoluene (0.10%)
- 0.035 kg butylated hydroxyanisole (0.35%)

- 1.2 kg Compound (1) free base (10%).
- 0.6 kg Polysorbate 80 (5%) weighed
- 10.153 kg oleic Acid (equivalent to 84.55 g (84.55%))

Solubilized crystalline compound (1) capsules, 20 mg or 40 mg, are manufactured through a series of unit process steps. Compound (1) drug substance, oleic acid, polysorbate 80, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) are mixed until a solution is achieved. The solution is filled into 2-piece hard gelatin capsules. Closed capsules are subsequently sealed with a hydroalcoholic solution, which is evaporated during the sealing process. A vacuum leak test is performed on sealed capsules prior to packaging.

Alternative Formulations

The crystalline compound of formula (1) optionally is used as an intermediate to be formulated into a solubilized form with the following agents:

- Fatty acids (short, medium, and long chained as well as saturated and unsaturated), typically C4 to C22. Typical fatty acids are linoleic acid, lauric acid, capric acid or oleic acid.
- Alcohols such as ethanol, benzyl alcohol, glycerol, polyethylene glycol 200, polyethylene glycol 300, polyethylene glycol 400.
- Surfactants, including both ionic and non-ionic surfactants. Examples of non-ionic surfactants are fatty acid esters of polyoxyethylene sorbitan, sorbitan fatty acid ester, polyoxyethylene castor oil derivatives, polyoxyethleneglycerol oxystearate, polyethyleneglycol 60, hydrogenated castor oil, and/or block copolymers of ethylene oxide and propylene oxide.

- Antioxidants, for example butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ascorbyl palmitate, vitamin E, and/or vitamin E PEG 1000 succinate for chemical stability.
- Viscosity inducer (silicon dioxide, polyethylene glycols, titanium oxide and the like).
- And mixtures of the above

Encapsulation can be performed in a soft elastic gelatin or a hard gelatin or a hard hydroxypropyl methyl cellulose capsule. The liquid formulation (solution or encapsulated solution) provides improved oral bioavailability.

Capsule Filling

The composition and preparation of the soft elastic gelatin capsule is well known in the art. The composition typically comprises from 30-50% by weight gelatin, 10-40% plasticizer or a blend of plasticizers and about 25-40% by weight water. Plasticizers can be glycerin, sorbitol or sorbitol derivatives, propylene glycol and the like or a combination thereof.

Various methods can be used for manufacturing and filling the soft elastic gelatin capsules such as rotary, liner or accogel machine and the like. Hard gelatin or HPMC capsules can be purchased from Capsugel, Greenwood, S.C. and other suppliers. Capsules are filled manually or by capsule filling machine.

Formulation Preparation

In general, the compositions of this invention can be prepared in the following manner. The ingredients are mixed in an appropriate vessel size using an overhead mixer (The mixing tank may be purged with nitrogen). The

pharmaceutically acceptable fatty acid and the pharmaceutically acceptable antioxidant are mixed at room temperature. (The solution may be warmed to appropriate temperature if needed, for example to about 45°C in the case of lauric acid, in order to liquefy the fatty acid). The compound of formula (1) is added and stirred until dissolved. The pharmaceutically acceptable surfactant is added with mixing. The appropriate weight of the resulting mixture is filled into hard gelatin capsules

Additional Formulation Compositions

Formula (1) compound	8.0
PEG 400	82.8
EtOH	9.2
Total	100.0

Formula (1) compound	8.0
EtOH	11.0
PG	7.4
Maisine 35-1	36.8
Cremophor RH40	36.8
Total	100.0

Formula (1) compound	8.0
Oleic Acid	92.0
Total	100.0

Formula (1) compound	8.0
Oleic Acid	73.6
EtOH	9.2
Tween 20	9.2
Total	100.0

Formula (1) compound	8.00%
Oleic Acid	87.40%
Tween 80	4.60%
Total	100.00%

FORMULA (1) COMPOUND	20.00%
Oleic Acid	80.0%
Total	100.0%

FORMULA (1)	
COMPOUND	20.00%
Oleic Acid	76.00%
Tween 80	4.00%
Total	100.00%

FORMULA (1)	
COMPOUND	8.00
Oleic Acid	86.47%
Tween 80	4.60%
Aerosil 200	0.92%
BHT	0.01%
Total	100.0%

FORMULA (1)	
COMPOUND	8.00
Oleic Acid	85.55%
Tween 80	4.60%
Aerosil 200	1.84%
BHT	0.01%
Total	100.0%

FORMULA (1)	
COMPOUND	8.00
Oleic Acid	85.55%
Tween 80	4.60%
Aerosil 200	1.84%
BHT	0.01%
Total	100.0%

FORMULA (1)	
COMPOUND	10.00
Oleic Acid	84.55%
Tween 80	5.00%
BHA	0.35%
BHT	0.1%
Total	100.0%

5

Example 2a

Micronized Formulation of Compound (1)

10 Micronized drug substance (Jet mill-00 at 60-80 psi; 3-4 microns average size, about 7-8 sq. meters/g) was dry blended with lactose, microcrystalline cellulose, croscarmellose sodium, sodium lauryl sulfate, tartaric acid, and hydroxypropyl cellulose. The blend was granulated by spraying the blend solution. The granules were dried in a fluid-bed. The dried granules were 15 sized by passing through a mill, and then blended with additional microcrystalline cellulose and croscarmellose sodium. The powder blend was lubricated by adding magnesium stearate and then compressed into tablets using a rotary tablet press. The tablets were subsequently film-coated.

20 The table below is a summary of various formulations tested in dogs dosed at 40 mg compound (1), corresponding to approximately 4 mg/kg. The table illustrates the superior performance of the solubilized compound (1) formulations.

In-vivo Data Summary

Dosage Form	Process	Formula	Drug Load (%)	Cmax (μM)	AUC_{24} (μM hr)	F (%)	RSD (%)
Solid	Powder Fill[a]	PIC	50	0.7	2.9	8	52
Solubilized	Liquid Fill	Capric acid	20	4.8	25	79	17
		Lauric acid	20	2.6	14.3	44	29
		Oleic Acid	8	3.8	23	67	27
			20	2.1	14	44	56
			25	7.9	42	125	24
Solid	High Shear[a]	SLS only	20	0.4	4.4	13	85
		SLS & Tartaric	20	0.4	2.7	8	82
		SLS & Tartaric[b]	20	0.9	6.9	20	67
	Fluid bed[a]	SLS & Tartaric	20	0.3	4.4	14	77

[a] Utilizes micronized API

[b] Dosed in dogs treated with pentagastrin to reduce stomach pH

Example 3

Antiviral Activity of Compound (1)

The compound of this invention exhibits anti-HCV replicon activity (assay described in WO 05/063744) against both genotypes 1a and 1, extremely low cytotoxicity (>50,000 nM in Huh-7, HepG2 and MT4 cells), and a highly favorable selectivity index. The compound is substantially less active against genotype 2a.

Activity of Compound 1 Against HCV Genotype 1 and 1a Replicons

HCV genotype 1 (Con-1/lucneo) and 1a (H77/neo) replicon cells were incubated with serial dilutions of compound (1) 2'C-methyl adenosine (2'CMeA) or IFNα for 3 days in the absence or presence of 40 mg/mL human serum albumin (HSA). After incubation, replicon RNA levels in the treated

cells were determined by either a luciferase reporter assay (1 replicon) or a quantitative real-time PCR assay (1a replicon) and the data points were used to calculate EC_{50} (50% effective inhibiting concentration) values for the inhibitors. Compound (1) was shown to inhibit both genotype 1 and genotype 1a replicons with EC_{50} values of 0.6 and 3.6 nM, respectively (Table A). In the presence of human serum albumin, the EC_{50} value of Compound (1) was increased to 11 nM.

Table A: Activity of Compound (1) against HCV Genotypes 1a and 1 Replicons

	EC_{50} [nM][a]		
Compound	HCV 1-lucneo	HCV 1-lucneo 40 mg/mL HSA	HCV-1a
1	0.6 ± 0.28	11	3.6 ± 1.4
2'CMeA	175 ± 70	250	170
IFN-α	2 IU/mL	n.d.	n.d.

n.d., not determined; HSA, human serum albumin

a Mean EC_{50} value and standard error determined from at least 4 independent experiments

Activity of Compound (1) Against HCV Genotype 1a Replicon and Virus

The antiviral activity of compound (1) against HCV genotype 2a was tested in cells chronically infected with the genotype 2a virus as well as in cells replicating a subgenomic 2a replicon. Huh-7 cells containing chronically replicating HCV genotype 2a (J6/JFH-Rluc) virus or subgenomic replicons were cultured with compound (1) or 2'CMeA for 3 days in the absence of human serum albumin. After cultivation, the amount of luciferase in 2a-virus containing cells and HCV NS3 protease activity in the 2a replicon-containing

cells was determined using Promega's luciferase assay and a novel time-resolved fluorescence assay, respectively.

The antiviral activity of compound (1) was significantly reduced in both the HCV-2a chronically infected cell culture model (EC_{50} = 2.9 μM) and the 2a subgenomic replicon model (EC_{50} = 21.9 μM) compared to Huh-7 cells replicating an HCV-1 subgenomic replicon (EC_{50} = 0.0006 μM) (Table 2). Taken together, these results suggest that the reduction in potency for compound (1) against HCV genotype 2a may be due to the genotypic differences between genotype 1 and genotype 2 of HCV.

Table B: Activity of Compound (1) against HCV Genotypes 1 and 2a

	EC_{50} [nM][a]		
Compound	HCV 1-lucneo (subgenomic replicon)	HCV 2a (subgenomic replicon)	HCV-2a (reporter virus)
1	0.6 ± 0.28	21898 ± 18972	2900 ± 1250
2'CMeA	175 ± 70	1610 ± 1099	194 ± 26
IFN-α	2 IU/mL	n.d.	1.2 IU/mL

n.d., not determined; HSA, human serum albumin

a Mean EC_{50} value and standard error determined from at least 4 independent experiments

Compound (1) was evaluated for its cytotoxicity in a variety of cell types including HCV replicon-containing cell lines (Huh-7, SL3 and MH4) and non-replicon-containing cell lines (HepG2, MT4), using a CellTiter-Glo Luminescence Cell Viability assay (Promega). No toxic effects were observed in any of the cell lines at the highest concentration tested (50 μM) (Table C). These results, coupled with its potent antiviral activity (EC_{50} = 0.62-3.6 nM) in HCV-1

and HCV-1a replicons, indicates a high selectivity index (CC_{50}/ EC_{50}>13,000-80,000) for compound (1).

Table C: Cytotoxicity of compound (1) in HCV Replicon Containing Cell Lines

	CC_{50} [μM][a]				
Compound	Huh-7 lucneo[b]	SL3[b]	MH4[b]	HepG2	MT4
1	> 50	> 50	> 50	> 50	> 50
2'CMeA	7.2 ± 6	3.9	16	24.3 ± 2.1	3.5 ± 1.9

n.d., not determined; HSA, human serum albumin

a Mean CC_{50} value and standard error determined from at least 4 independent experiments
b HCV replicon-containing cell lines

Anti-HCV Activity of Compound (1) in Combination with IFN In Vitro

Pegylated interteron-α (PEG-IFN-α), in combination with ribavirin, represents the current standard of care for HCV-infected patients. *In vitro* combination studies of compound (1) and IFN-α were performed in replicon cells. Data was analyzed using the MacSynergy template developed by Prichard and Shipman. Results from these studies suggest an additive interaction between compound (1) and IFN-α.

Example 4

Antiviral, Pharmacokinetic and Safety Data for Compound (1) in a Phase-1, First-In-Human Trial in HCV Genotype 1-Infected Subjects.

A randomized, double-blind, placebo controlled trial was designed to evaluate the safety/tolerability, phamacokinetics and antiviral activity of single (in Part A) and multiple (in Part B) doses of Compound (1) (oleic acid solution,

above) in subjects chronically infected with HCV genotype 1 (GT-1) without decompensated cirrhosis. Prospective subjects are 18-60 years of age, are HCV treatment naïve, and are in general good health.

In completed Part A, five successive cohorts of 6 subjects were randomized (5:1) to receive single ascending doses of Compound 1 (40, 120, 240, 240-with food, or 480 mg) or placebo. In ongoing Part B, four successive cohorts of 12 subjects are randomized (10:2) to receive multiple ascending doses of Compound 1 (40 mg BID, 120 mg BID, 240 mg QD, 240 mg BID) or placebo, over 8 days.

Thirty-one subjects enrolled in Part A were of mean age 43.6 years, predominantly male (20/31), Caucasian (25/31), and infected with either HCV Genotype-1a (24) or 1 (6). Median (range) baseline HCV viral load was 6.6 Log^{10} RNA IU/mL (5.2-7.3). Single doses of compound (1) were well tolerated, with no serious or treatment-limiting adverse events (AEs) reported. The most common AE was headache. All AEs were mild in severity, with the exception of one moderate headache. There were no Grade 3 or 4 treatment emergent laboratory abnormalities.

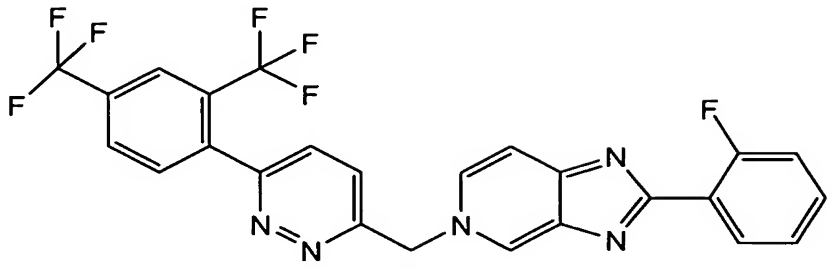
Median compound (1) plasma half-life ranged from 10 to 15 hours across cohorts. Systemic exposure was increased approximately 2-fold when compound (1) was administered with a high fat meal. Mean compound (1) concentration 24 hours after the 240 mg fasted dose dosing was ~7-fold higher than the protein binding adjusted *in vitro* HCV GT-1 Replicon EC_{50} value. Following single-dose exposure, maximal antiviral effect was observed at 24 hours, with median declines ranging from 0.46 to 1.49 Log^{10} HCV RNA IU/mL

across cohorts. Individual HCV RNA declines among all compound (1) recipients ranged from 0.19 to 2.54 $\log^{10}$ IU/mL following single-dose exposure.

This is the first clinical demonstration of antiviral activity of compound (1). Single dose exposure to compound (1) was well tolerated, demonstrated favorable PK properties and potent antiviral activity.

We claim:

1. A crystalline compound of formula (1)



(1)

and its salts, which is substantially free of amorphous compound (1) .

2. The crystalline compound of claim 1 having an endothermic onset at about 235°C in differential scanning calorimetry (DSC) profile.

3. The crystalline compound of claim 2 having a heat of fusion (DH_f) of about 81 J/g (42 KJ/mole).

4. The crystalline compound of claim 3 having at least one approximate peak at diffraction angle 2θ (º) of about 17 as measured by X-ray powder diffractometry.

5. The crystalline compound of claim 1 which is the free base.

6. The crystalline compound of claim 1 in the form of needles or rods.

7. A composition comprising the crystalline compound of claim 1 containing less than about 40% by weight of the amorphous compound (1).

8. A composition comprising the crystalline compound of claim 7 which contains less than about 10% by weight of amorphous compound (1).

9. A composition comprising the crystalline compound of claim 8 wherein the crystalline compound (1) contains less than about 100 ppm chloride.

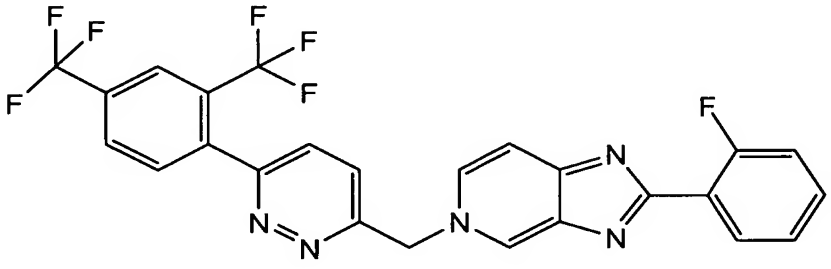
10. The crystalline compound of claim 1 which has been micronized.

11. The crystalline compound which is the free base substantially free of amorphous compound (1) and any other crystal form of compound (1).

12. The crystalline compound of claim 1 which is substantially free of the chloride salt of compound (1).

13. A composition comprising the crystalline compound of claim 1 and a pharmaceutically acceptable excipient.

14. A method for making crystalline compound (1)



comprising crystallizing compound (1) from crystallization solvent and controlling the amount of water in the crystallization solvent.

15. The method of claim 14 wherein the compound (1) is crystallized from ethyl acetate or ethyl acetate/isopropyl alcohol solvent containing less than about 0.9% by weight of water in the solvent.

16. The method of claim 14 wherein the amount of water is controlled such that less than about 10% by weight of amorphous compound (1) is precipitated during crystallization.

17. The method of claim 14 wherein the water is controlled by removing it azeotropically from the crystallization solvent.

18. The method of claim 14 comprising providing water in the crystallization solvent at a concentration of less than about 10%.

19. The method of claim 18 comprising a plurality of crystallization steps, wherein crystallization is effected from solvents comprising successively lower concentrations of water.

20. The method of claim 18 wherein the last crystallization step is effected from solvent comprising less than about 0.9% water.

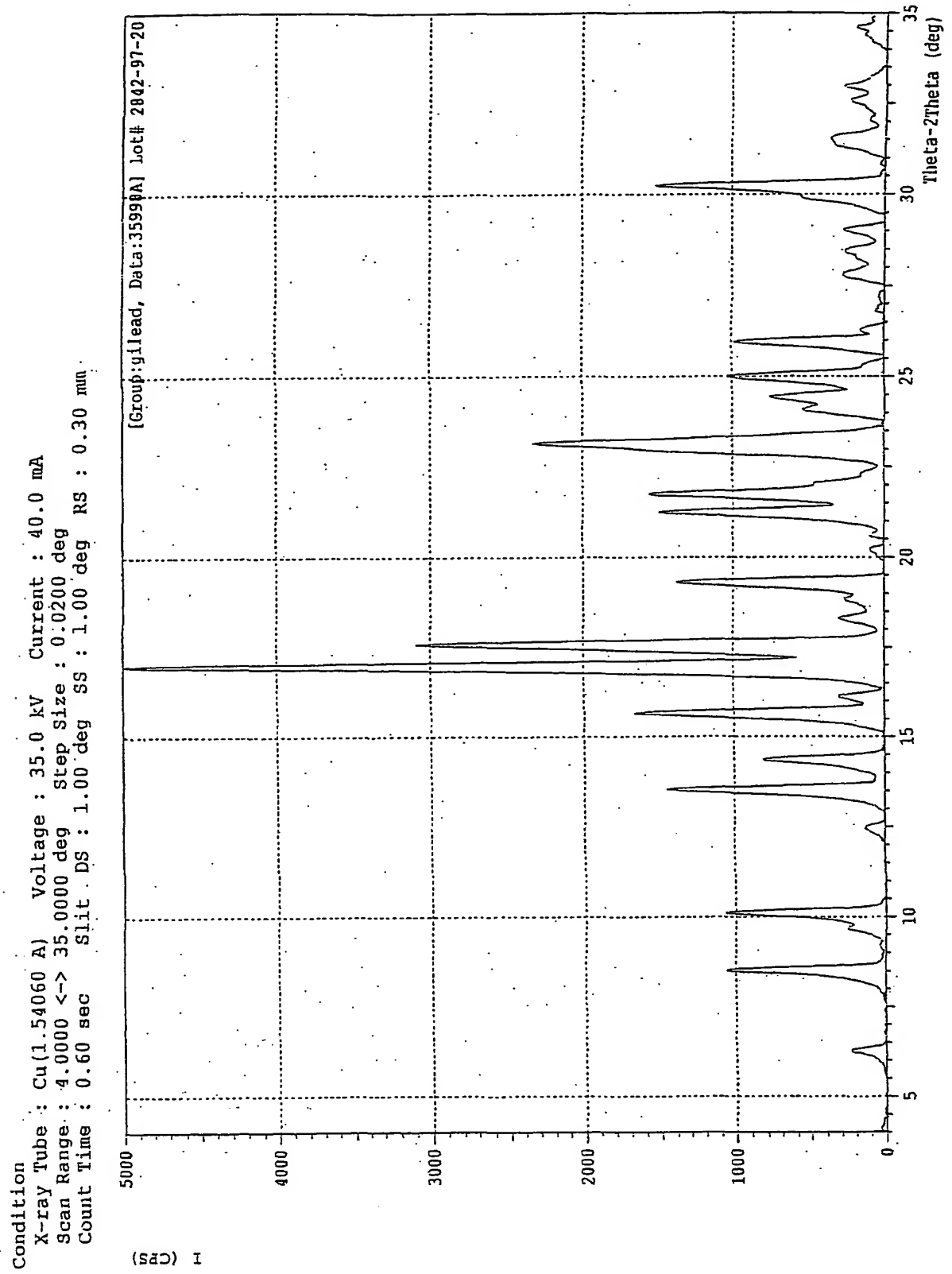

[Group:gilead, Data:35990A] Lot# 2842-97-20
Condition
X-ray Tube : Cu(1.54060 A) Voltage : 35.0 kV Current : 40.0 mA
Scan Range : 4.0000 <-> 35.0000 deg Step Size : 0.0200 deg
Count Time : 0.60 sec Slit DS : 1.00 deg SS : 1.00 deg RS : 0.30 mm
I (CPS)
5000
4000
3000
2000
1000
0
5
10
15
20
25
30
35
Theta-2Theta (deg)

Figure 1/5

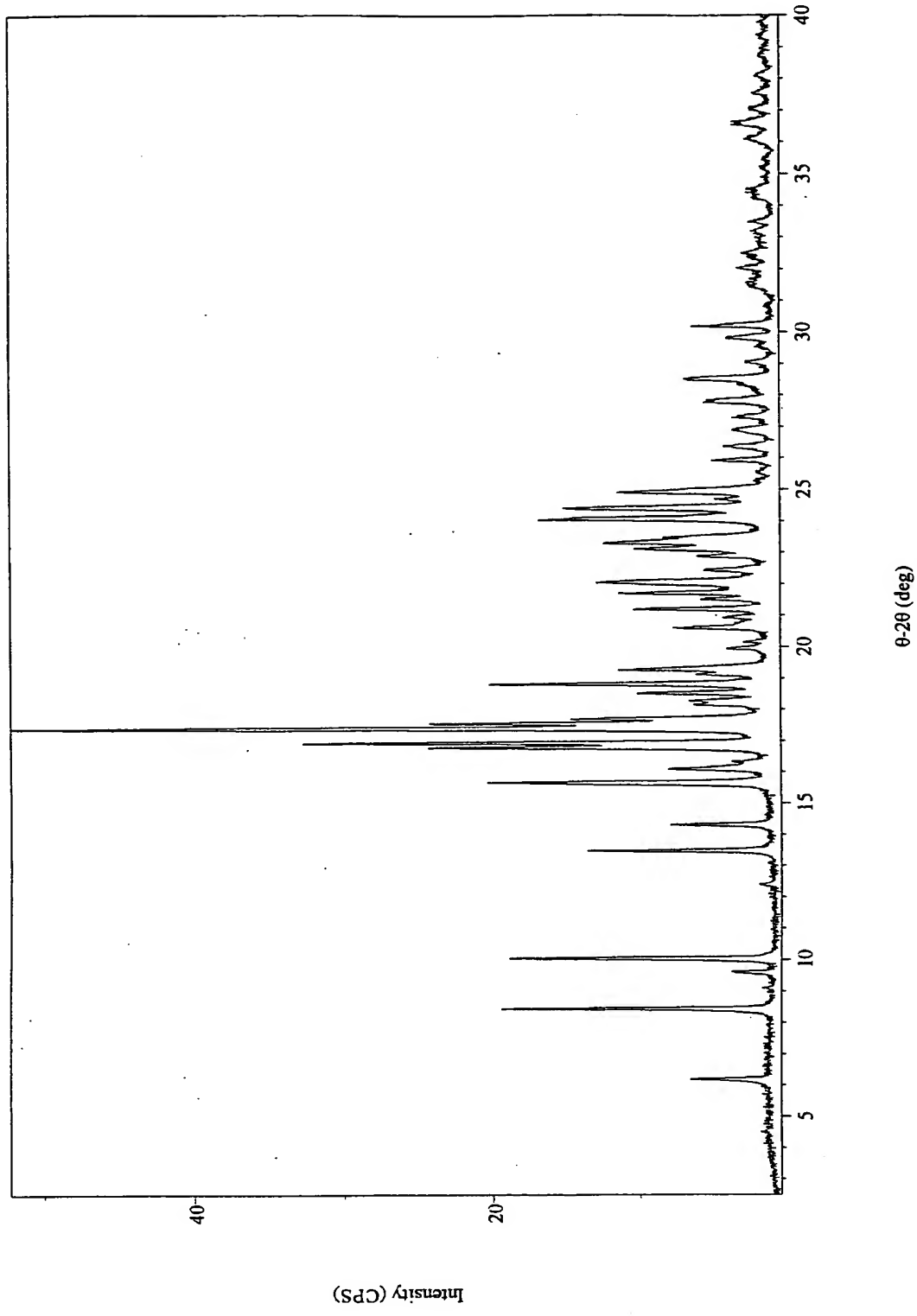
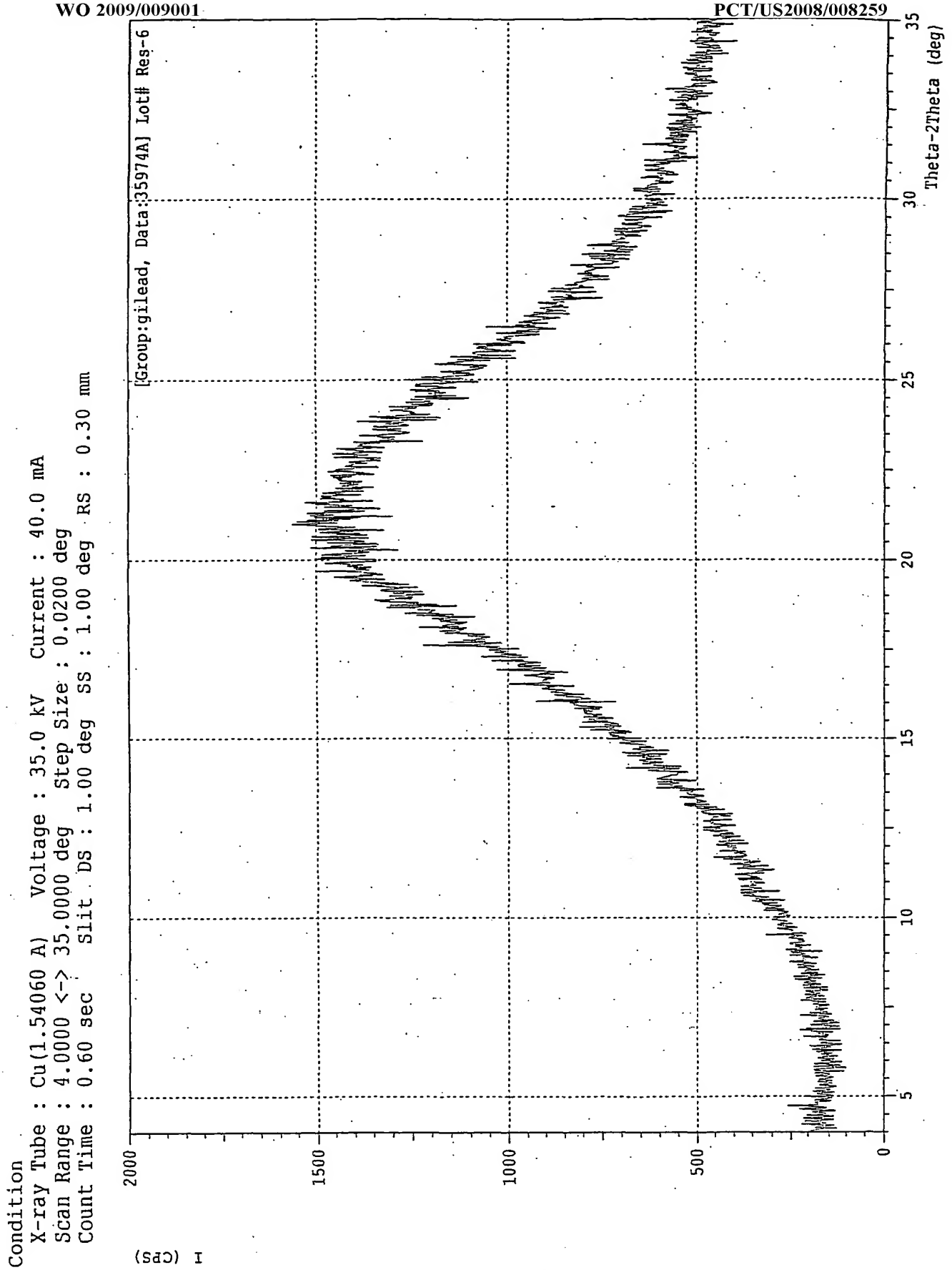


Figure 2/5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Count Time : 0.60 sec Slit DS : 1.00 deg SS : 1.00 deg RS : 0.30 mm
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0
5
10
15
20
25
30
35
Theta-2Theta (deg)

Figure 3/5

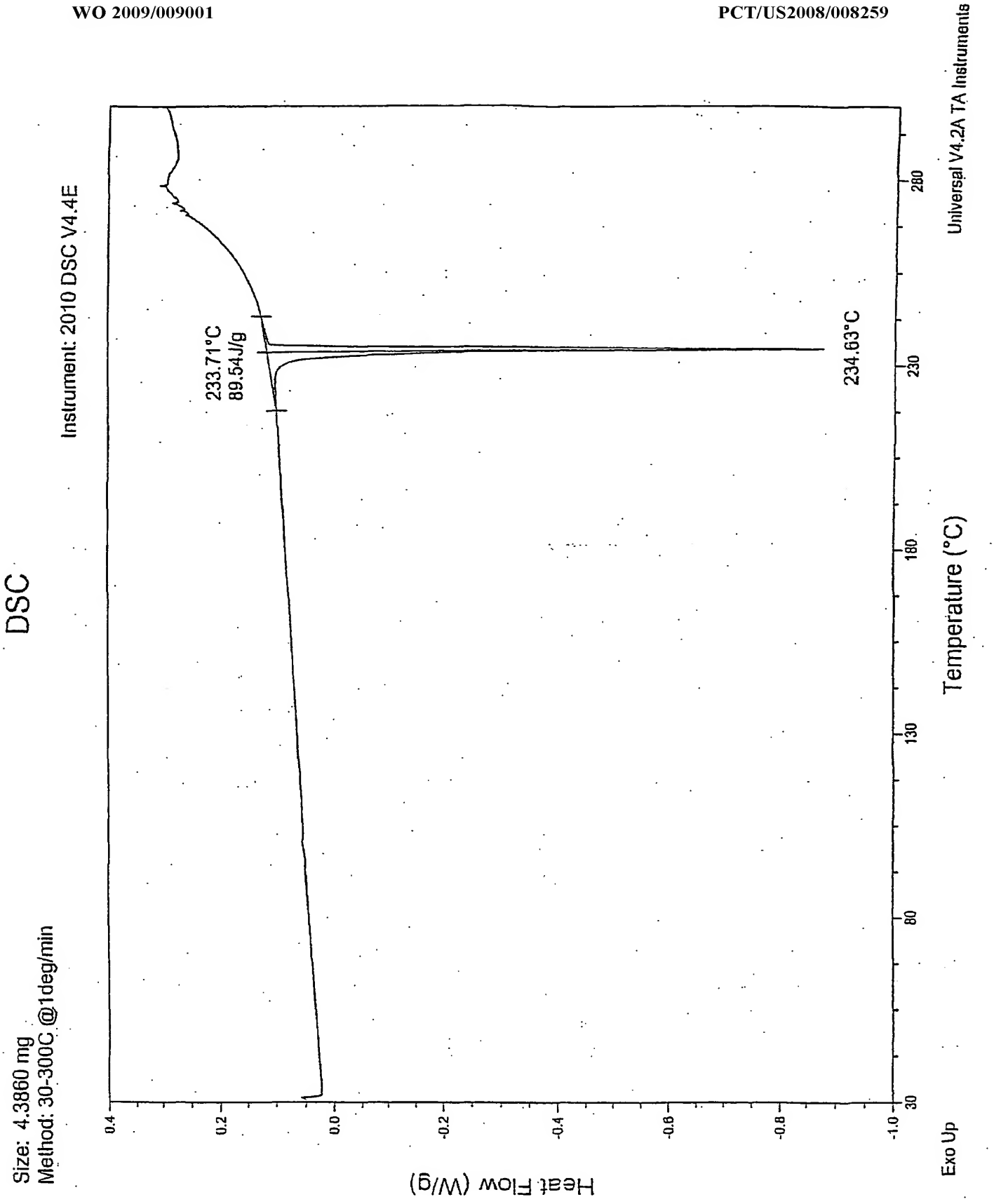
DSC
Instrument: 2010 DSC V4.4E
Size: 4.3860 mg
Method: 30-300C @1deg/min
233.71°C
89.54J/g
234.63°C
Heat Flow (W/g)
Temperature (°C)
Exo Up
Universal V4.2A TA Instruments

Figure 4/5

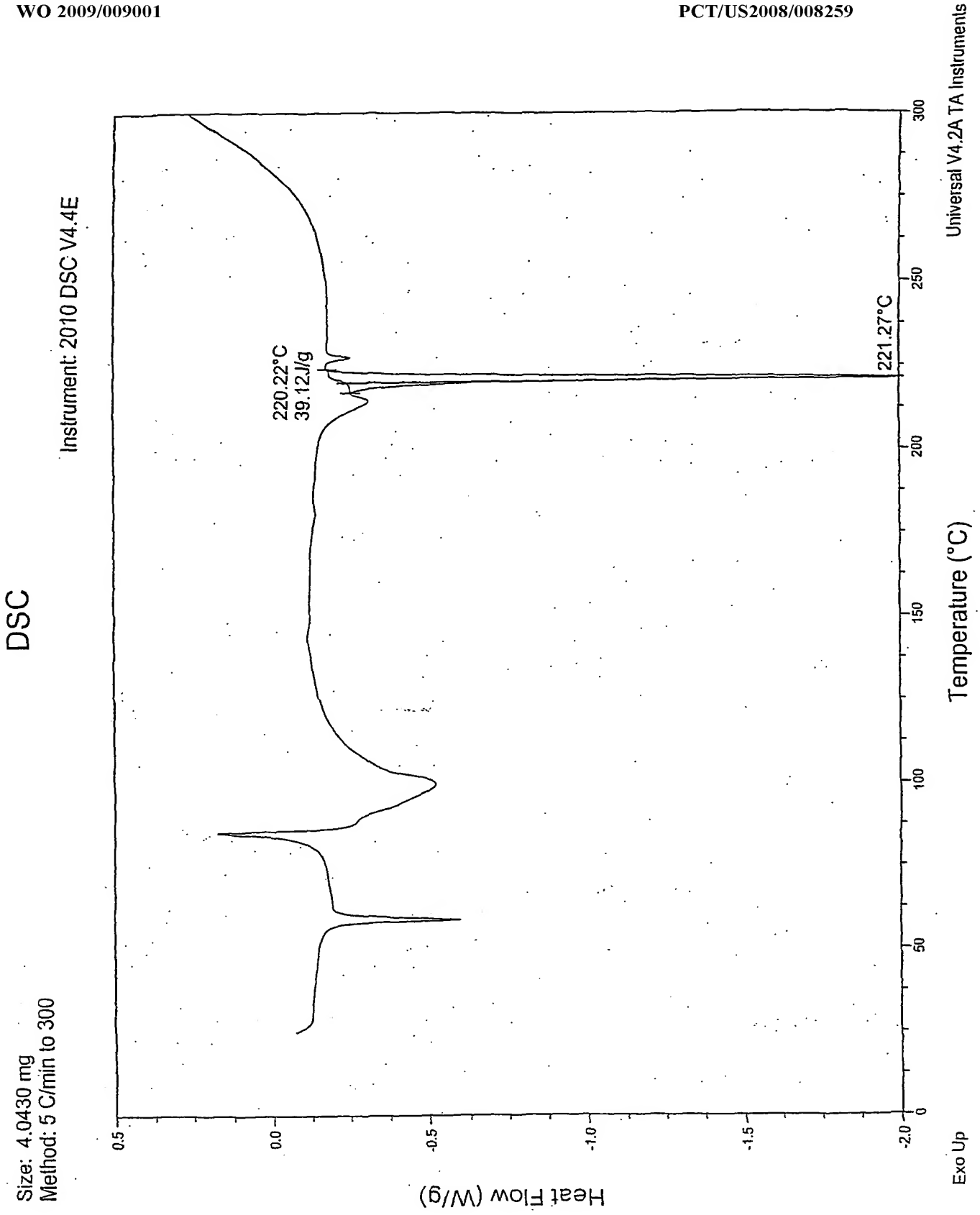
DSC
Instrument: 2010 DSC V4.4E
Size: 4.0430 mg
Method: 5 C/min to 300
220.22°C
39.12J/g
221.27°C
Heat Flow (W/g)
Temperature (°C)
Exo Up
Universal V4.2A TA Instruments

Figure 5/5

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/008259

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D471/04 A61K31/4353 A61P31/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005/063744 A (LEUVEN K U RES & DEV [BE]; PUERSTINGER GERHARD [AT]; GILEAD SCIENCES I) 14 July 2005 (2005-07-14) cited in the application example 317	1-20
P,X	WO 2008/005519 A (GILEAD SCIENCES INC [US]; LEUVEN K U RES & DEV [BE]; PUERSTINGER GERHA) 10 January 2008 (2008-01-10) cited in the application example 1b	1-20

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 October 2008	14/10/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Fax: (+31–70) 340–3016	Usuelli, Ambrogio

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2008/008259

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005063744 A	14-07-2005	AU 2004309390 A1	14-07-2005
		CA 2549606 A1	14-07-2005
		EP 1706403 A2	04-10-2006
		JP 2007518720 T	12-07-2007
		KR 20060132850 A	22-12-2006
		US 2005222198 A1	06-10-2005
		US 2007244148 A1	18-10-2007
WO 2008005519 A	10-01-2008	US 2008199427 A1	21-08-2008

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(54) Title: NOVEL 3-FUSED PYRIDINIUMMETHYL CEPHALOSPORINS

(57) Abstract

Novel semi-synthetic cephalosporin derivatives having a fused pyridiniummethyl at 3-position of the cephem nucleus, pharmaceutically acceptable salts, physiologically hydrolizable esters or solvates thereof are disclosed. Also disclosed is a process for preparing the cephalosporin derivatives which comprises introducing a fused pyridiniummethyl substituent at 3-position of 7-β-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamino]-3-cephem-carboxylic acid derivatives. The compounds of the present invention show potent antibacterial activities and a broad spectrum against both gram-positive and gram-negative bacterial.

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NOVEL 3-FUSED PYRIDINIUMMETHYL CEPHALOSPORINS

TECHNICAL FIELD

The present invention relates to novel cephalosporin derivatives, a pharmaceutically acceptable salt, and physiologically hydrolyzable ester and solvate thereof. This invention also relates to a process for their preparation, a use thereof as an antibiotic, and a pharmaceutical composition containing the same derivatives as an active ingredient.

BACKGROUND ART

A number of cephalosporin compounds have been synthesized in which the cephem nucleus has a quarternary ammonium methyl at its 3-position and various acylamino groups at its 7-position. These compounds exhibit selective toxicity against bacteria only and present no substantial effects against animal cells. They have been widely used for the treatment of infectious diseases caused by bacteria as antibiotics having no substantial side effects. Thus, they are highly useful as drugs.

In recent years, an extensive investigation has been made to develop novel cephalosporin derivatives which have more potent antibacterial activities and a broad antibacterial spectrum, especially coupled with activities against cephalosporin resistant bacteria.

As a result, a number of cephalosporin derivatives have been developed which have a 2-(2-aminothiazol-4-yl)-2-substituted oxyiminoacetamido group as a side chain at 7-

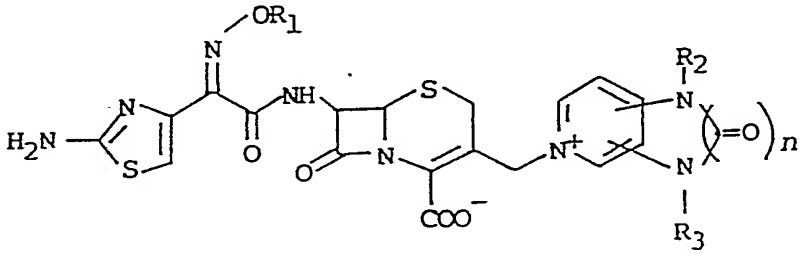
position and a fused pyridiniummethyl substituted at 3-position of the cephem nucleus. As prior art references which disclose such derivatives, U.S. Patent No. 4,152,432 to Heymes et al., U.S. Patent No. 4,098,888 to Ochiai et al., U.S. Patent No. 4,258,041 to O'Callaghan, U.S. Patent No. 4,748,172 to Katner, European Patent No. 0,138,552 to Katner, European Patent No. 0,164, 944 to Bradbury, and European Patent No. 0,300,664 to Jung may be mentioned.

The present invention has been accomplished as an advanced improvement as compared with such investigation.

Thus, the object of the invention is to provide novel cephalosporin derivatives having strong activities and a broad antibacterial spectrum against both gram-positive and gram-negative bacteria, as well as excellent stability against ß-lactamase.

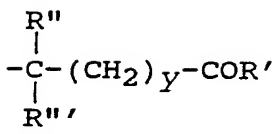
DISCLOSURE OF THE INVENTION

The present invention provides novel cephalosporin derivatives having the formula:



(I)

wherein R_1 is hydrogen, or a lower alkyl, C_3-C_4 alkenyl, C_3-C_4 alkynyl or cycloalkylalkyl group, a fluoro-substituted lower alkyl group represented by the formula: $-(CH_2)_xF$ in which x is an integer of 1 to 3, or a carboxy-substituted alkyl group represented by the formula:



wherein R′ is a hydroxy, amino or C_1-C_4 alkoxy group; R" and R"′, which may be the same or different, represent hydrogen or a C_1-C_3 alkyl group, or R" and R"′ together with the carbon atom to which they are attached may form a C_3-C_7 carbocyclic ring; and *y* is an integer of 0 to 3;

R_2 and R_3, which may be the same or different, represent hydrogen, or a lower alkyl, amino, carboxy-substituted lower alkyl, hydroxy-substituted lower alkyl or C_3-C_7 cycloalkyl group;

n is an integer of 1 or 2; and

the 2-oxo-heterocyclic moiety is fused with the pyridine ring to form a 2,3- or 3,4-fused ring substituent at 3-position of the cephem nucleus; or a pharmaceutically acceptable salt, physiologically hydrolyzable ester or solvate thereof.

The compounds of the present invention show strong activities against gram-positive bacteria such as *Streptococcus*, *Staphylococcus*, Methicillin resistant *Staphylococcus*, *Corynebacterium*, *Bacillus*, etc.; gram-negative bacteria such as *Escherichia*, *Enterobacter*, *Klebsiella*, *Serratia*, *Salmonella*, *Proteus*, *Providensia*, *Morganella*, *Pseudomonas*, etc.; and various drug resistant bacteria.

Particularly preferred specific compounds according to the invention are as set forth below:

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacet-

amido]-3-[2,3(1H,4H)-dioxo-pyrazino[5,6-c]pyridinium-methyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-ethoxyiminoacetamido]-3-[2,3(1H,4H)-dioxo-pyrazino[5,6-c]pyridinium-methyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-aminothiazol-4-yl)-2-propynyloxyiminoacetamido]-3-[2,3(1H,4H)-dioxo-pyrazino[5,6-c]pyridinium-methyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-cyclopropylmethoxyiminoacetamido]-3-[2,3(1H,4H)-dioxo-pyrazino[5,6-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-carboxymethoxyiminoacetamido]-3-[2,3(1H,4H)-dioxo-pyrazino[5,6-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-yl)oxyiminoacetamido]-3-[2,3(1H,4H)-dioxo-pyrazino-[5,6-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[1-methyl-2,3(4H)-dioxo-pyrazino[5,6-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[1-ethyl-2,3(4H)-dioxo-pyrazino[5,6-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[1-cyclopropyl-2,3(4H)-dioxo-pyrazino[5,6-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-yl)oxyiminoacetamido]-3-[1-methyl-2,3(4H)-dioxo-pyrazino[5,6-c]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-yl)oxyiminoacetamido]-3-[1-ethyl-2,3(4H)-dioxo-pyrazino[5,6-c]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-yl)oxyiminoacetamido]-3-[1-cyclopropyl-2,3(4H)-dioxo-pyrazino[5,6-c]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[4-methyl-2,3(1H)-dioxo-pyrazino[5,6-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(aminothiazol-4-yl)-2-fluoromethoxyiminoacetamido]-3-[2,3(1H,4H)-dioxo-pyrazino[5,6-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[2(1H,3H)-oxo-imidazo[4,5-c]pyridinium-methyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[1-methyl-2(3H)-oxo-imidazo[4,5-c]pyridinium-methyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[1-amino-2(3H)-oxo-imidazo[4,5-c]pyridinium-methyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[1-(2-hydroxyethyl)-2(3H)-oxo-imidazo[4,5-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[2(1H,3H)-oxo-imidazo[4,5-b]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-fluoromethoxyiminoacetamido]-3-[2(1H,3H)-oxo-imidazo[4,5-c]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-fluoromethoxyiminoacetamido]-3-[1-methyl-2(3H)-oxo-imidazo[4,5-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-fluoromethoxyiminoacetamido]-3-[1-amino-2(3H)-oxo-imidazo[4,5-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-fluoromethoxyiminoacetamido]-3-[2(1H,3H)-oxo-imidazo[4,5-b]pyridiniummethyl]-3-cephem-4-carboxylate; and

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-carboxymethoxyiminoacetamido]-3-[2(1H,3H)-oxo-imidazo[4,5-c]-pyridiniummethyl]-3-cephem-4-carboxylate.

The new cephalosporin compounds of the present invention may be in the form of either a *syn*- or *anti*-isomer, or a mixture thereof consisting of at least about 90 % of a *syn*-isomer and not more than 10 % of an *anti*-isomer.

Also, if R_1 is a carboxy-substituted alkyl group

represented by the formula: -C(R")(R"')COOH wherein R" and R"' are different from each other, then the carbon atom to which R" and R"' are linked may be an asymmetrical center, resulting in diastereoisomers. Therefore, the present invention also includes such diastereoisomers of the cephalosporin derivatives of the formula (I) above, and mixtures thereof.

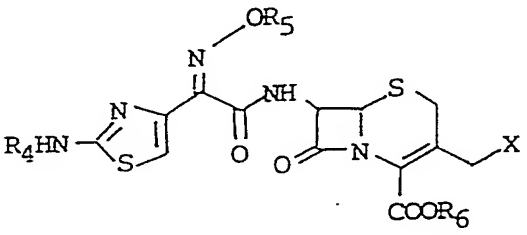
The compounds of the formula (I) may be converted to non-toxic salts thereof by conventional methods. Such non-toxic salts may be pharmaceutically acceptable salts of the compound of the formula (I). Included among the non-toxic salts are an inorganic salt, for example, a metal salt such as an alkali metal salt (e.g., sodium salt, potassium salt, etc.), an alkaline earth metal salt (e.g., calcium salt, magnesium salt, etc.), ammonium salt, and so forth; an organic salt, for example, an organic amine salt (e.g., trimethylamine salt, triethylamine salt, pyridine salt, procaine salt, picoline salt, decyclohexylamine salt, N,N-dibenzylethylenediamine salt, N-methyl glucamine salt, diethanolamine salt, triethanolamine salt, tris(hydroxy-methylamino)methane salt, phenylethylbenzylamine salt, dibenzylethylenediamine salt, and so forth; an organic carboxylic or sulfonic acid salt (e.g., formate, acetate, maleate, tartrate, methanesulfonate, benzenesulfonate, toluenesulfonate, etc.); an inorganic acid salt (e.g., hydrochloride, hydrobromide, sulfate, phosphate, etc.); a salt with a basic or acidic amino acid (e.g., arginine, aspartic acid, glutamic acid, lysine, etc.); and the like.

The physiologically hydrolyzable esters of the compounds of the formula (I) may include, for example, indanyl, phthalidyl, methoxymethyl, pivaloyloxymethyl, glycyloxymethyl, phenylglycyloxymethyl or 5-methyl-2-oxo-1,3-dioxolan-4-yl esters, and other physiologically

hydrolyzable esters which have been widely used in penicillin and cephalosporin antibiotics chemistry.

The present invention further provides a process for preparing the novel cephalosporin derivatives of the formula (I) comprising the steps of:

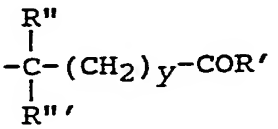
reacting a compound of the formula:



(II)

wherein R_4 is an amino protecting group;

R_5 is hydrogen, or a lower alkyl, C_3-C_4 alkenyl, C_3-C_4 alkynyl or cycloalkylalkyl group, a fluoro-substituted lower alkyl represented by the formula: $-(CH_2)_xF$, in which x is an integer of 1 to 3, or a carboxy-substituted alkyl group represented by the formula:

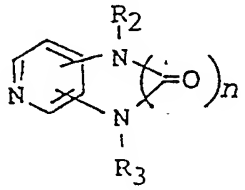


wherein R′ is a hydroxy, amino or C_1-C_4 alkoxy group; R" and R"′ may be the same or different and represent hydrogen or a C_1-C_3 alkyl group, or R" and R"′ together with the carbon atom to which they are attached may form a C_3-C_7 carbocyclic ring; and y is an integer of 0 to 3;

R_6 is a carboxyl protecting group; and

X is a leaving group;

with a compound of the formula:



(III)

wherein R_2, R_3 and n have the same meaning as defined above and the 2-oxo-heterocyclic moiety is fused with the pyridine ring to form a 2,3- or 3,4-fused ring; and then, if necessary, removing the amino protecting group and/or the carboxyl protecting group.

In the preparation of the objective compounds of the formula (I), the compound of the formula (II) is preferably used in an amount of from 1 to 2 equivalents based on 1 equivalent of the compound of the formula (III).

Now, the symbols and terms used in the specification will be explained.

The term "lower" as used herein above and elsewhere in this specification, for example, with reference to "lower alkyl," means those group having 1 to 6, preferably 1 to 4 carbon atoms.

The amino protecting group may include an acyl group; a substituted or unsubstituted aryl-lower alkyl group, for example, benzyl, diphenylmethyl, triphenylmethyl and 4-methoxybenzyl; a halo-lower alkyl group, for example, trichloromethyl and trichloroethyl; tetrahydropyranyl; a substituted phenylthio group; a substituted alkylidene group; a substituted aralkylidene group; and a substituted cyclolidene group. The acyl group as an amino protecting

group may include, for example, a C_1-C_6 alkanoyl group such as formyl and acetyl; a C_2-C_6 alkoxy carbonyl group, for example, methoxycarbonyl and ethoxycarbonyl; a lower alkane sulfonyl group, for example, methane sulfonyl and ethane sulfonyl; or an aryl-lower alkoxy carbonyl group such as benzyloxycarbonyl. One to three substituents such as a halogen atom, or a hydroxy, cyano or nitro group can further be substituted for the acyl group. In addition, the amino protecting group may include the reaction products formed by a reaction of an amino group with silane, boron, or phosphorous compounds.

The carboxyl protecting group such as R_6 may include, for example, a lower alkyl group such as methyl and t-butyl; a lower alkenyl group such as vinyl and allyl; a lower alkoxy-lower alkyl group such as methoxymethyl; a lower alkylthio-lower alkyl group such as methylthiomethyl; a halo-lower alkyl group such as 2,2,2-trichloroethyl; a substituted or unsubstituted aralkyl group such as benzyl and p-nitrobenzyl; or a silyl group.

The amino or carboxyl protecting groups mentioned above may be readily removed under mild conditions by using a known method(See: *Protecting Groups in Organic Synthesis*, 3rd Ed.).

The leaving group, X, may include, for example, a halogen atom such as fluorine, chlorine, and iodine; a lower alkanoyloxy group such as acetoxy; a lower alkanesulfonyloxy group such as methanesulfonyloxy; an arenesulfonyloxy group such as p-toluenesulfonyloxy; an alkoxy carbonyloxy group; and the like.

BEST MODE FOR CARRYING OUT THE INVENTION

The displacement reaction of the compound of the formula (II) with the compound of the formula (III) is well performed when X is an acetoxy group or an iodine atom.

In an embodiment, a compound of the formula (II) in which X is an acetoxy group is first silylated with a silylating agent to protect the carboxy group at 4-position and the amino group of the substituent at 7-position. As the silylating agent, mono- or bis-trimethylsilylacetamide, N-methyl-N-(trimethylsilyl)acetamide, N,O-bis(trimethylsilyl)trifluoroacetamide, N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) and hexamethyldisilazane (HMDS) may be used.

The silylated compound of the formula (II) is then reacted with trimethylsilyliodide (TMSI) at ambient temperature to form a compound of the formula (II) in which X is iodine. This reaction can be carried out in accordance with a known method, for example, as taught by U.S. Patent No. 4,266,049 to Bonjouklian.

Separately, the fused pyridine of the formula (III) is silylated at room temperature in an aprotic organic solvent using the same silylating agent as mentioned above.

The resulting silylated 3-iodomethyl cephalosporin of the formula (II) is then reacted with the silylated fused pyridine of the formula (III) to give a silylated compound of the formula (I). Hydrolysis of the silyl groups provides a compound of the formula (I) according to the present invention.

The reaction for introducing the substituent of the

formula (III) at 3-position of the compound of the formula (II) to prepare the compound of the formula (I) is carried out in the presence of an organic solvent such as an anhydrous aprotic solvent. As an appropriate organic solvent, there may be mentioned a nitrile solvent such as acetonitrile and propionitrile; an alkyl halide solvent such as chloroform, carbon tetrachloride and dichloromethane; an ether solvent such as tetrahydrofuran and dioxane; an amide solvent such as N,N-dimethyl formamide; an ester solvent such as ethylacetate and methylacetate; a ketone solvent such as acetone, methyl ethyl ketone and methyl isobutyl ketone; a sulfoxide solvent such as dimethylsulfoxide; and an aromatic carbohydrogen solvent such as benzene and toluene. This reaction may be carried out at 0 ^{o}C to 25 ^{o}C.

In an alternative embodiment, the compounds of the formula (I) according to the invention are prepared directly from a 3-acetoxymethyl compound, for example, a compound of the formula (II) in which X is an acetoxy and R_4 is H.

This reaction is carried out in a conventional manner, for instance, in an aqueous medium, for example in an organic solvent in admixture with water. Addition of a small amount of an alkali iodide such as potassium iodide can enhance the rate of the reaction. This reaction is carried out at a temperature between about 35 ^{o}C and about 70 ^{o}C. Useful water miscible organic solvents include acetone, acetonitrile, tetrahydrofuran, and dimethylacetamide.

However, it is preferred to use the former method, i.e., reacting a compound of the formula (II) in which X is iodine with a compound of the formula (III) in view of the

reactivity and yields.

The amino or acid protecting groups can be readily removed by a conventional deprotection method well known in cephalosporin antibiotics chemistry. For example, acid- or base-hydrolysis or reduction are generally applicable. For example, when the protecting group is an amido group, such compound is subjected to imino-halogenation and imino-etherification, followed by hydrolysis. Acid hydrolysis is preferably applicable to the removal of the groups such as tri(di)-phenylmethyl or alkoxycarbonyl. As a preferred acid for this purpose, there may be mentioned organic acids such as formic acid, trifluoroacetic acid and p-toluene-acetic acid; or an inorganic acid such as hydrochloric acid and the like.

During and after the preparation, a stabilizing agent can be used to stabilize reaction products and their intermediates. As a stabilizing agent, one or more salts selected from the group consisting of sodium iodide, potassium iodide, sodium bromide, potassium bromide and potassium thiocyanate can be mentioned.

The compounds of the formula (I) have the same stereo-chemistry as the known cephalosporin antibiotics. That is, the side chain at 7-position has a ß-configuration (6R,7R), while the oxyimino group in the side chains may be in either a *syn*- or *anti*-form, or as a mixture thereof. Thus, the compounds of the present invention are prepared in either form by employing the 2-(heterocyclic)-2-oxyimino-acetic acid in the *syn*- or *anti*-form and coupling reagents. Instead, separation and purification of the compounds of the formula (I) can be performed by means of recry-stallization, column chromatography, or ion exchange chromatography.

The present invention also provides a pharmaceutical composition comprising, as an active ingredient, one or more of the compounds of the formula (I) according to the present invention, a non-toxic salt, physiologically hydrolyzable ester or solvate thereof, in association with pharmaceutically acceptable carriers, excipients, or other additives.

The antibiotic compounds of the formula (I), as well as a non-toxic salt, physiologically hydrolyzable ester or solvate thereof may be formulated for administration, which may be presented in an unit dose form or in a multidose container. The formulation may be in various forms such as solutions, suspensions, or emulsions in oily or aqueous vehicles, which can contain conventional additives such as dispersing agents, suspending agents, stabilizing agents, and the like. In addition, the compounds of the present invention may be formulated into a dried powder that can be normally dissolved in an aqueous solution of sterile, pyrogen-free water, prior to use. The compounds of the present invention may also be formulated into a suppository containing conventional suppository bases such as cocoa and other glycerides.

PREFERRED EMBODIMENT OF THE INVENTION

The present invention will be described in greater detail by way of the following examples. The examples are presented for illustration purpose only and should not be construed as limiting the invention which is properly delineated in the claims.

PREPARATION 1: PREPARATION OF 2,3(1H,4H)-DIOXO-PYRAZINO-[5,6-c]PYRIDINE

To a solution of 4 g of 3,4-diaminopyridine in 120 ml of methanol, 4.36 g of sodium methoxide was added, and the mixture was stirred at room temperature for 30 minutes. A solution of 4.3 g of dimethyloxalate in 40 ml of methanol was added dropwise to the mixture over 30 minutes and the resulting mixture heated to reflux for 7 hours. The mixture was concentrated under reduced pressure, diluted with 240 ml of water, and then cooled in an ice bath. The reaction mixture was adjusted to pH 6.5 with 10 % hydrochloric acid. The precipitated solids were collected by filtration, washed with water, and dried to give 4.5 g of the title compound as a white solid.

IR (KBr, cm^{-1}) : 3230; 1709; 1383.

NMR (DMSO-d_6) : 12.1(2H,s); 8.4(1H,s); 8.2(1H,d); 7.05 (1H,d).

PREPARATION 2: PREPARATION OF 1-METHYL-2,3(4H)-DIOXO-PYRAZINO[5,6-c]PYRIDINE

3-Amino-4-methylaminopyridine was reacted in the manner similar to that described in Preparation 1 to give the title compound.

IR (cm^{-1}): 3433; 1707; 1420.

NMR (DMSO-d_6): 12.1(1H,s); 8.4(1H,s); 8.3(1H,d); 7.4(1H,d); 3.5(3H,s).

PREPARATION 3: PREPARATION OF 4-METHYL-2,3(1H)-DIOXO-PYRAZINO[5,6-c]PYRIDINE

3-Methylamino-4-aminopyridine was reacted in the manner similar to that described in Preparation 1 to give the title compound.

IR (KBr, cm^{-1}): 3225; 1708; 1380.

NMR (DMSO-d_6): 8.55(1H,s); 8.26(1H,d); 7.09(1H,d); 3.53(3H,s).

PREPARATION 4: PREPARATION OF 1-ETHYL-2,3(4H)-DIOXO-PYRAZINO[5,6-c]PYRIDINE

3-Amino-4-ethylaminopyridine was reacted in the manner similar to that described in Preparation 1 to give the title compound.

IR (cm^{-1}): 1703; 1612; 1391.

NMR (DMSO-d_6): 12.1(1H,s); 8.4(1H,s); 8.3(1H,d); 7.4(1H,d); 4.0(2H,q); 1.2(3H,t).

PREPARATION 5: PREPARATION OF 1-CYCLOPROPYL-2,3(4H)-DIOXO-PYRAZINO[5,6-c]PYRIDINE

3-Amino-4-cyclopropylaminopyridine was reacted in the manner similar to that described in Preparation 1 to give the title compound.

IR (cm^{-1}): 1707; 1612; 1416.

NMR (DMSO-d_6): 12.1(1H,s); 8.4(1H,s); 8.3(1H,d); 7.4(1H,d); 3.5(1H,m); 0.5(4H,m).

PREPARATION 6: PREPARATION OF 2(1H,3H)-OXO-IMIDAZO[4,5-c]-PYRIDINE

A mixture of 3 g of 3,4-diaminopyridine, 1.65 g of urea, and 30 ml of N,N-dimethylformamide was heated to reflux for 6 hours. The reaction mixture was cooled to room temperature and stirred for 12 hours. The precipitated solids were collected by filtration and dissolved in 30 ml of methanol. The resultant solution was treated with active carbon and evaporated under reduced pressure to give 3.1 g of the title compound as a white solid.

m.p. : 315°C (decomp.)

IR (KBr, cm^{-1}) : 3125; 1717; 1630.

NMR (DMSO-d_6) : 8.14(1H,s); 8.10(1H,d, J=5.19Hz); 6.97 (1H,d, J=5.19Hz).

PREPARATION 7: PREPARATION OF 1-METHYL-2(3H)-OXO-IMIDAZO-[4,5-c]PYRIDINE

3-Amino-4-methylaminopyridine was reacted in the manner similar to that described in Preparation 6 to give the title compound.

m.p. : 263-265 °C.

IR (KBr, cm^{-1}) : 2739; 1715; 1624.

NMR (D_2O) : 8.18(1H,s); 8.13(1H,d, J=5.3Hz); 7.1(1H,d, J=5.3Hz); 3.27(3H,s).

PREPARATION 8: PREPARATION OF 1-AMINO-2(3H)-OXO-IMIDAZO-[4,5-c]PYRIDINE HYDROCHLORIDE

3-Amino-4-hydrazinopyridine was reacted in the manner similar to that described in Preparation 6 to give the title compound.

m.p. : 309-310 °C (decomp.)

IR (KBr, cm^{-1}) : 3236; 3144; 3077; 1739; 1723.

NMR (D_2O) : 7.55-8.45(2H,2d, J=6.0Hz); 8.49(1H,s); 9.50(NH,bs); 12.5(NH_2,bs).

PREPARATION 9: PREPARATION OF 1-(2-HYDROXYETHYL)-2(3H)-OXO-IMIDAZO[4,5-c]PYRIDINE

3-Amino-4-(2-hydroxyethyl)aminopyridine was reacted in the manner similar to that described in Preparation 6 to give the title compound.

IR (KBr, cm^{-1}) : 3400; 3144; 1740; 1715.

NMR (D_2O) : 7.55-8.45(2H,2d); 8.5(1H,S); 9.5(NH,bs); 3.3-3.5(4H,dd).

PREPARATION 10: PREPARATION OF 2(1H,3H)-OXO-IMIDAZO[4,5-b]-PYRIDINE

2,3-Diaminopyridine was reacted in the manner similar to that described in Preparation 6 to give the title compound.

m.p. : 270-272 °C.

IR (KBr, cm^{-1}) : 3462; 1692; 1434.

NMR (DMSO-d_6) : 11.2(1H,s); 10.71(1H.s); 7.85(1H,s, J=1.6, 5.1Hz); 7.20(1H,s, J=1.6, 7.7Hz); 3.33(3H,s).

EXAMPLE 1: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-METHOXYIMINOACETAMIDO]-3-[2,3(1H,4H)-DIOXO-PYRAZINO[5,6-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

To a suspension of 500 mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid in 10 ml of well-dried dichloromethane was added, in one portion, 0.80 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide at room temperature. The reaction mixture was stirred for 5 minutes at 25 °C under a nitrogen atmosphere. To the stirred solution was added by pipette 0.50 ml of iodotrimethylsilane at 0 °C, and the reaction mixture was then stirred at room temperature for 30 minutes. Thereafter, the solvent was evaporated off under reduced pressure to provide an oil. The oil was dissolved in a mixture of 10 ml of acetonitrile and 1.0 ml of tetrahydrofuran, and the solution was stirred for 5 minutes. The stirred solution was added, in one portion, to a solution of 180 mg of 2,3(1H,4H)-dioxo-pyrazino[5,6-c]pyridine silylated with 0.80 ml of N,O-bis-(trimethylsilyl)acetamide in 10 ml of acetonitrile. The reaction mixture was stirred for 3 hours at 25 °C and then added to a mixture of 1.0 ml of methanol and 2 ml of acetonitrile at 0 °C. The mixture was stirred at 0 °C for 30 minutes. The precipitated solids were collected by filtration to give a solid product. 10 Ml of water was

added to the solid, and the mixture was neutralized with saturated sodium bicarbonate solution and then concentrated. The residue was purified by chromatography over silica gel to give 100 mg of the title compound.

m.p. : 210 °C (decomp.)

IR (KBr, cm^{-1}) : 1771; 1685; 1618.

NMR (DMSO-d_6) : 9.55(1H,d); 8.5(2H,m); 7.4(1H,d); 6.9(1H,s); 5.85(1H,dd,J=6Hz); 5.1(1H,d,J=6Hz); 3.8(3H,s)

EXAMPLE 2: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-ETHOXYIMINOACETAMIDO]-3-[2,3(1H,4H)-DIOXO-PYRAZINO[5,6-c]-PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

510 Mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-ethoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid was suspended in 10 ml of dry dichloromethane and reacted with 0.8 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide and then 0.5 ml of iodotrimethylsilane in the same manner as described in Example 1. The reaction mixture was concentrated. The concentrate was dissolved in a mixture of 10 ml of acetonitrile and 1 ml of tetrahydrofuran to give a solution. Separately, 200 mg of 2,3(1H,4H)-dioxo-pyrazino[5,6-c]pyridine was reacted with 0.8 ml of N,O-bistrimethylsilylacetamide in 5 ml of acetonitrile to give a silylated pyridine derivative, which was then added to the solution previously obtained. The mixture was reacted at room temperature for 3 hours. Then, to the reaction mixture, 1 ml of methanol was added to effect deprotection. The precipitated solids were filtered out and purified to give 200 mg of the title compound.

m.p.: 208 °C (decomp.)

IR (cm^{-1}): 1773; 1687; 1620.

NMR (DMSO-d_6): 9.55(1H,d); 8.5(2H,m); 7.4(1H,d); 6.9(1H,s); 5.85(1H,dd,J=6Hz);

5.1(1H,d,J=6Hz); 4.4(2H,q); 1.4(3H,t).

EXAMPLE 3: SYNTHESIS OF 7-ß-[(Z)-2-AMINOTHIAZOL-4-YL)-2-PROPYNYLOXYIMINOACETAMIDO]-3-[2,3(1H,4H)-DIOXO-PYRAZINO-[5,6-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

500 Mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-propynyloxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid was suspended in 10 ml of dry dichloromethane and reacted with 0.8 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide and then 0.5 ml of iodotrimethylsilane in the same manner as described in Example 1. The reaction mixture was concentrated. The concentrate was dissolved in a mixture of 10 ml of acetonitrile and 1 ml tetrahydrofuran to give a solution. Separately, 200 mg of 2,3(1H,4H)-dioxo-pyrazino[5,6-c]pyridine was reacted with 0.8 ml of N,O-bistrimethylsilylacetamide in 5 ml of acetonitrile to give a silylated pyridine derivative, which was then added to the solution previously obtained. The mixture was reacted at room temperature for 3 hours. Then, to the reaction mixture, 1 ml of methanol was added to effect deprotection. The precipitated solids were filtered out and purified to give 210 mg of the desired compound.

m.p.: 220 ^{o}C (decomp.)

IR (cm^{-1}): 1773; 1690; 1620.

NMR (DMSO-d_6): 9.6(1H,d); 8.55(2H,m); 7.4(1H,d); 6.9(1H,s); 5.8(1H,dd,J=6Hz); 5.1(1H, d,J=6Hz); 4.7(2H,m)

EXAMPLE 4: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-CYCLOPROPYLMETHOXYIMINOACETAMIDO]-3-[2,3(1H,4H)-DIOXO-PYRAZINO[5,6-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

540 Mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-cyclopropylmethoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-

carboxylic acid was suspended in 10 ml of dry dichloromethane and reacted with 0.8 ml of N-methyl-N-(trimethylsilyl)-trifluoroacetamide and then 0.5 ml of iodotrimethylsilane in the same manner as described in Example 1. The reaction mixture was concentrated. The concentrate was dissolved in a mixture of 10 ml of acetonitrile and 1 ml tetrahydrofuran to give a solution. Separately, 200 mg of 2,3(1H,4H)-dioxo-pyrazino[5,6-c]pyridine was reacted with 0.8 ml of N,O-bistrimethylsilylacetamide in 5 ml of acetonitrile to give a silylated pyridine derivative, which was then added to the solution previously obtained. The mixture was reacted at room temperature for 3 hours. Then, to the reaction mixture, 1 ml of methanol was added to effect deprotection. The precipitated solids were filtered out and purified to give 230 mg of the title compound.

m.p.: 215 °C (decomp.)

IR (cm^{-1}): 1774; 1690.

NMR (DMSO-d_6): 9.6(1H,d); 8.55(2H,m); 7.4(1H,d); 6.9(1H,s); 5.8(1H,dd,J=6Hz); 5.1(1H, d,J=6Hz); 4.3(2H,d); 0.5-1.0(4H,m).

EXAMPLE 5: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-CARBOXYMETHOXYIMINOACETAMIDO]-3-[2,3(1H,4H)-DIOXO-PYRAZINO-[5,6-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

530 Mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-carboxymethoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid was suspended in 15 ml of dry dichloromethane and reacted with 1 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide and then 0.5 ml of iodotrimethylsilane in the same manner as described in Example 1. The reaction mixture was concentrated. The concentrate was dissolved in a mixture of 15 ml of acetonitrile and 1 ml of tetrahydrofuran to give a solution. Separately, 200 mg of 2,3(1H,4H)-dioxo-pyrazino[5,6-c]pyridine was reacted with

0.8 ml of N,O-bistrimethylsilylacetamide in 5 ml of acetonitrile to give a silylated pyridine derivative, which was then added to the solution previously obtained. The mixture was reacted at room temperature for 3 hours. Then, to the reaction mixture, 1 ml of methanol was added to effect deprotection. The precipitated solids were filtered out and purified to give 250 mg of the title compound.

m.p.: 218 °C (decomp.)

IR(cm^{-1}): 1772; 1687.

NMR(DMSO-d_6): 9.6(1H,d); 8.5(2H,m); 7.4(1H,d); 6.9 (1H,s); 5.8(1H,dd,J=6Hz); 5.1(1H,d, J=6Hz); 4.6(2H,s).

EXAMPLE 6: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-(2-CARBOXYPROP-2-YL)OXYIMINOACETAMIDO]-3-[2,3(1H,4H)-DIOXO-PYRAZINO[5,6-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

560 Mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-yl)oxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid was suspended in 15 ml of dry dichloromethane and reacted with 1 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide and then 0.5 ml of iodotrimethylsilane in the same manner as described in Example 1. The reaction mixture was concentrated. The concentrate was dissolved in a mixture of 15 ml of acetonitrile and 1 ml of tetrahydrofuran to give a solution. Separately, 200 mg of 2,3(1H,4H)-dioxo-pyrazino[5,6-c]pyridine was reacted with 0.8 ml of N,O-bistrimethylsilylacetamide in 5 ml of acetonitrile to give a silylated pyridine derivative, which was then added to the solution previously obtained. The mixture was reacted at room temperature for 3 hours. Then, to the reaction mixture, 1 ml of methanol was added to effect deprotection. The precipitated solids

were filtered out and purified to give 250 mg of the title compound.

m.p.: 220 °C (decomp.)

IR(cm^{-1}): 1773; 1692.

NMR(DMSO-d_6): 9.55(1H,d); 8.55(2H,m); 7.4(1H,d); 6.9 (1H,s); 5.8(1H,dd,J=6Hz); 5.1(1H,d, J=6Hz); 1.5(6H,s).

EXAMPLE 7: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-METHOXYIMINOACETAMIDO]-3-[1-METHYL-2,3(4H)-DIOXO-PYRAZINO[5,6-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

500 Mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxy-iminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid was suspended in 10 ml of dry dichloromethane was reacted with 0.8 ml of N-methyl-N-(trimethylsilyl)tri-fluoroacetamide and then 0.5 ml of iodotrimethylsilane in the same manner as described in Example 1. The reaction mixture was concentrated. The concentrate was dissolved in a mixture of 10 ml of acetonitrile and 1 ml of tetrahydrofuran to give a solution. Separately, 240 mg of 1-methyl-2,3(4H)-dioxo-pyrazino[5,6-c]pyridine was reacted with 0.8 ml of N,O-bistrimethylsilylacetamide in 5 ml of acetonitrile to give a silylated pyridine derivative, which was then added to the solution previously obtained. The mixture was reacted at room temperature for 3 hours. Then, to the reaction mixture, 1 ml of methanol was added to effect deprotection. The precipitated solids were filtered out and purified to give 250 mg of the title compound.

m.p.: 205 °C (decomp.)

IR(cm^{-1}): 1775; 1714.

NMR(DMSO-d_6): 9.6(1H,d); 8.5(2H,m); 7.4(1H,d); 6.9 (1H,s); 5.8(1H,dd,J=6Hz); 5.15(1H,d, J=6Hz); 3.8(3H,s); 3.5(3H,s).

EXAMPLE 8: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-METHOXYIMINOACETAMIDO]-3-[1-ETHYL-2,3(4H)-DIOXO-PYRAZINO-[5,6-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

500 Mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid was suspended in 10 ml of dry dichloromethane and reacted with 0.8 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide and then 0.5 ml of iodotrimethylsilane in the same manner as described in Example 1. The reaction mixtue was concentrated. The concentrate was dissolved in a mixture of 10 ml of acetonitrile and 1 ml of tetrahydrofuran to give a solution. Separately, 250 mg of 1-ethyl-2,3(4H)-dioxo-pyrazino[5,6-c]pyridine was reacted with 0.8 ml of N,O-bistrimethylsilylacetamide in 5 ml of acetonitrile to give a silylated pyridine derivative, which was then added to the solution previously obtained. The mixture was reacted at room temperature for 3 hours. Then, to the reaction mixture, 1 ml of methanol was added to effect deprotection. The precipitated solids were filtered out and purified to give 250 mg of the title compound.

m.p.: 210 °C (decomp.)

IR(cm^{-1}): 1775; 1716.

NMR(DMSO-d_6): 9.6(1H,d); 8.5(2H,m); 7.4(1H,d); 6.9 (1H,s); 5.8(1H,dd,J=6Hz); 5.2(1H,d, J=6Hz); 3.8(3H,s); 4.0(2H,q); 1.2(3H,t).

EXAMPLE 9: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-METHOXYIMINOACETAMIDO]-3-[1-CYCLOPROPYL-2,3(4H)-DIOXO-PYRAZINO[5,6-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

500 Mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid was suspended in 10 ml of dry dichloromethane was

reacted with 0.8 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide and then 0.5 ml of iodotrimethylsilane in the same manner as described in Example 1. The reaction mixture was concentrated. The concentrate was dissolved in a mixture of 10 ml of acetonitrile and 1 ml of tetrahydrofuran to give a solution. Separately, 270 mg of 1-cyclopropyl-2,3(4H)-dioxo-pyrazino[5,6-c]pyridine was reacted with 0.8 ml of N,O-bistrimethylsilylacetamide in 5 ml of acetonitrile to give a silylated pyridine derivative, which was then added to the solution previously obtained. The mixture was reacted at room temperature for 3 hours. Then, to the reaction mixture, 1 ml of methanol was added to effect deprotection. The precipitated solids were filtered out and purified to give 230 mg of the title compound.

m.p.: 208 ^{o}C(decomp.)

IR(cm^{-1}): 1774; 1716.

NMR(DMSO-d_6): 9.6(1H,d); 8.5(2H,m); 7.4(1H,d); 6.9 (1H,s); 5.8(1H,dd,J=6Hz); 5.2(1H,d, J=6Hz); 3.8(3H,s); 3.5(1H,m), 0.6(4H,m).

EXAMPLE 10: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-(2-CARBOXYPROP-2-YL)OXYIMINOACETAMIDO]-3-[1-METHYL-2,3(4H)-DIOXO-PYRAZINO[5,6-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

560 Mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-yl)oxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid was suspended in 15 ml of dry dichloromethane and reacted with 1 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide and then 0.5 ml of iodotrimethylsilane in the same manner as described in Example 1. The reaction mixture was concentrated. The concentrate was dissolved in a mixture of 15 ml of acetonitrile and 1 ml of tetrahydrofuran to give a solution. Separately, 240 mg

of 1-methyl-2,3(4H)-dioxo-pyrazino[5,6-c]pyridine was reacted with 0.8 ml of N,O-bistrimethylsilylacetamide in 5 ml of acetonitrile to give a silylated pyridine derivative, which was then added to the solution previously obtained. The mixture was reacted at room temperature for 3 hours. Then, to the reaction mixture, 2 ml of methanol was added to effect deprotection. The precipitated solids were filtered out and purified to give 200 mg of the title compound.

m.p.: 215 °C (decomp.)

IR(cm^{-1}): 1773; 1715.

NMR(DMSO-d_6): 9.6(1H,d); 8.55(2H,m); 7.4(1H,d); 6.9 (1H,s); 5.8(1H,dd,J=6Hz); 5.2(1H,d, J=6Hz); 3.5(3H,s); 1.5(6H,s).

EXAMPLE 11: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-(2-CARBOXYPROP-2-YL)OXYIMINOACETAMIDO]-3-[1-ETHYL-2,3(4H)-DIOXO-PYRAZINO[5,6-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

560 Mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-yl)oxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid was suspended in 15 ml of dry dichloromethane and reacted with 1 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide and then 0.5 ml of iodotrimethylsilane in the same manner as described in Example 1. The reaction mixture was concentrated. The concentrate was dissolved in a mixture of 15 ml of acetonitrile and 1 ml of tetrahydrofuran to give a solution. Separately, 250 mg of 1-ethyl-2,3(4H)-dioxo-pyrazino[5,6-c]pyridine was reacted with 0.8 ml of N,O-bistrimethylsilylacetamide in 5 ml of acetonitrile to give a silylated pyridine derivative, which was then added to the solution previously obtained. The mixture was reacted at room temperature for 3 hours. Then, to the reaction mixture, 2 ml of methanol

was added to effect deprotection. The precipitated solids were filtered out and purified to give 230 mg of the title compound.

m.p.: 217 °C (decomp.)

IR(cm^{-1}): 1774; 1717.

NMR(DMSO-d_6): 9.6(1H,d); 8.5(2H,m); 7.4(1H,d); 6.9 (1H,s); 5.8(1H,dd,J=6Hz); 5.2(1H,d, J=6Hz); 4.0(2H,q); 1.5(6H,s); 1.2(3H,t).

EXAMPLE 12: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-(2-CARBOXYPROP-2-YL)OXYIMINOACETAMIDO]-3-[1-CYCLOPROPYL-2,3(4H)-DIOXO-PYRAZINO[5,6-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

560 Mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-yl)oxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid was suspended in 15 ml of dry dichloromethane and reacted with 1 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide and then 0.5 ml of iodotrimethylsilane in the same manner as described in Example 1. The reaction mixture was concentrated. The concentrate was dissolved in a mixture of 15 ml of acetonitrile and 1 ml of tetrahydrofuran to give a solution. Separately, 270 mg of 1-cyclopropyl-2,3(4H)-dioxo-pyrazino[5,6-c]pyridine was reacted with 0.8 ml of N,O-bistrimethylsilylacetamide in 5 ml of acetonitrile to give a silylated pyridine derivative, which was then added to the solution previously obtained. The mixture was reacted at room temperature for 3 hours. Then, to the reaction mixture, 2 ml of methanol was added to effect deprotection. The precipitated solids were filtered out and purified to give 250 mg of the title compound.

m.p.: 215 °C (decomp.)

IR(cm^{-1}): 1775; 1716.

NMR(DMSO-d_6): 9.6(1H,d); 8.5(2H,m); 7.4(1H,d);

6.9(1H,s); 5.8(1H,dd,J=6Hz); 5.2(1H,d, J=6Hz); 3.5(1H,m); 1.5(6H,s); 0.6(4H,m).

EXAMPLE 13: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-METHOXYIMINOACETAMIDO]-3-[4-METHYL-2,3(1H)-DIOXO-PYRAZINO[5,6-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

To a suspension of 340 mg of 7-ß-[(z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid in 10 ml of dry dichloromethane was added, in one portion, 0.5 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide at room temperature. The reaction mixture was stirred for 5 minutes at 25 °C under a nitrogen atmosphere. To the stirred solution was added 0.24 ml of iodotrimethylsilane at 0 °C and the reaction mixture was then stirred at room temperature for 30 minutes. The solvent was removed by evaporation under reduced pressure to give an oil. The oil was dissolved in a mixture of 10 ml of acetonitrile and 1 ml of tetrahydrofuran. The resultant solution was stirred for 5 minutes. The stirred solution was added, in one portion, to a solution of 4-methyl-2,3(1H)-dioxo-pyrazino[5,6-c]pyridine silylated with 0.8 ml of N,O-bis(trimethylsilyl)acetamide in 10 ml of acetonitrile. The reaction mixture was stirred for 3 hours at 25 °C and then added to a mixture of 1.0 ml of methanol and 2 ml of acetonitrile at 0 °C. The precipitated solids were collected by filtration to give a solid product. 10 Ml of water was added to the solid, and the mixture was neutralized with a saturated $NaHCO_3$ solution and then concentrated. The resultant residue was purified by chromatography over silica gel eluting with acetonitrile:H_2O (4:1) and concentrated to give 40 mg of the title compound.

m.p.: 220 °C (decomp.)

IR (KBr, cm^{-1}): 1760; 1620.

NMR (DMSO-d_6): 9.7(1H,d,J=7.8Hz); 8.5(1H,s); 8.3 (1H,d); 7.10(1H,d); 6.8(1H,s); 5.6 (1H,dd, J=7.8, 4.5Hz); 5.1(1H,d, J=4.86Hz); 4.9(2H,bs); 3.75(3H,s); 3.5 (3H,s); 3.4(2H,m).

EXAMPLE 14: SYNTHESIS OF 7-ß-[(Z)-2-(AMINOTHIAZOL-4-YL)-2-FLUOROMETHOXYIMINOACETAMIDO]-3-[2,3(1H,4H)-DIOXO-PYRAZINO-[5,6-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

To a suspension of 700 mg of 7-ß-[(z)-2-(2-amino-thiazol-4-yl)-2-fluoromethoxyiminoacetamido]-3-acetoxy-methyl-3-cephem-4-carboxylic acid in 15 ml of dry dichloro-methane was added, in one portion, 1.0 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide at room temperature. The reaction mixture was stirred for 5 minutes at 25 °C under a nitrogen atmosphere. To the stirred solution was added by pipette 0.44 ml of iodotrimethylsilane at 0 °C, and the reaction mixture was then stirred at room temperature for 30 minutes. Thereafter, the mixture was evaporated under reduced pressure to remove the solvent and then provide an oil. The oil was dissolved in a mixture of 10 ml of acetonitrile and 1.0 ml of tetrahydrofuran, and the solution was stirred for 5 minutes. The stirred solution was added in one portion to a solution of 400 mg of 2,3(1H,4H)-dioxo-pyrazino[5,6-c]pyridine silylated with 0.88 ml of N,O-bis(trimethylsilyl)acetamide in 10 ml of acetonitrile. The reaction mixture was stirred for 3 hours at 25 °C and then added to a mixture of 1.0 ml of methanol and 2 ml of acetonitrile at 0 °C. The mixture was stirred at 0 °C for 30 minutes. The precipitated solids were collected by filtration to give a solid product. 10 Ml of water was added to the solid, and the mixture was neutralized with a saturated sodium bicarbonate solution and then concentrated. The residue was purified by

chromatography over silica gel to give 250 mg of the title compound.

m.p.: 220 °C (decomp.)

IR (KBr, cm^{-1}) 1770; 1688; 1619.

NMR (DMSO-d_6): 9.72(1H,bd,J=7.84Hz); 8.51(1H,s); 8.35 (1H,bs); 7.10(1H,d,J=5.7Hz); 6.88 (1H,s); 6.32(2H,d,J=55.18Hz); 5.65 (1H,dd, J=7.84, 4.87Hz); 5.05(1H,d, J=4.87Hz); 4.95(2H,bs); 3.44(2H,m).

EXAMPLE 15: SYNTHESIS OF 7-β-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-METHOXYIMINOACETAMIDO]-3-[2(1H,3H)-OXO-IMIDAZO[4,5-c]-PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

To a suspension of 500 mg of 7-β-[(Z)-2-(2-amino-thiazol-4-yl)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid in 10 ml of dry dichloromethane was added, in one portion, 0.7 ml of N-methyl-N-(trimethyl-sily)trifluoroacetamide at room temperature. The reaction mixture was stirred for 5 minutes at 25 °C under a nitrogen atmosphere. To the stirred solution was added by pipette 0.38 ml of iodotrimethylsilane at 0 °C, and the reaction mixture was then stirred at room temperature for 30 minutes. Thereafter, the reaction mixture was evaporated under reduced pressure to remove the solvent and then give an oil. The oil was dissolved in a mixture of 10 ml of acetonitrile and 1 ml of tetrahydrofuran. The resultant solution was stirred for 5 minutes. The stirred solution was added, in one portion, to a solution of 10 mg of 2(1H,3H)-oxo-imidazo[4,5-c]pyridine silylated with 0.62 ml of N,O-bis(trimethylsilyl)acetamide in 10 ml of aceto-nitrile. The reaction mixture was stirred for 3 hours at 25 °C and then added to a mixture of 0.5 ml of methanol and 5 ml of acetonitrile at 0 °C. The precipitated solids were collected by filtration to provide a solid product. 10 Ml

of water was added to the solid, and the mixture was neutralized with a saturated $NaHCO_3$ solution and then concentrated. The resultant residue was purified by chromatography over silica gel eluting with acetonitrile: H_2O (4:1) and concentrated to give 170 mg of the title compound.

m.p. : 219 °C (decomp.)

IR(KBr, cm^{-1}) : 1772, 1653, 1636.

NMR (DMSO-d_6) : 3.18(1H,d, J=17.1Hz); 3.53(1H, m); 3.58(1H,d, J=17.1Hz); 3.78(3H,s); 4.77(1H,m); 5.06(1H,d); 5.61(1H,dd); 6.70(1H,s); 7.18(1H,bd,J=6.85Hz); 8.48(1H,bd, J=6.85Hz); 8.85(1H,s); 9.48(1H,dd).

EXAMPLE 16: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-METHOXYIMINOACETAMIDO]-3-[1-METHYL-2(3H)-OXO-IMIDAZO[4,5-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

To a suspension of 450 mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid in 10 ml of dry dichloromethane was added, in one portion, 0.5 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide at room temperature. The reaction mixture was stirred for 5 minutes at 25 °C under a nitrogen atmosphere. To the stirred solution was added by pipette 0.24 ml of iodotrimethylsilane at 0 °C, and the reaction mixture was then stirred at room temperature for 30 minutes. Thereafter, the mixture was evaporated under reduced pressure to remove the solvent and then give an oil. The oil was dissolved in a mixture of 10 ml of acetonitrile and 1 ml of tetrahydrofuran. The resultant solution was stirred for 5 minutes. The stirred solution was added, in one portion, to a solution of 90 mg of 1-methyl-2(3H)-oxo-imidazo[4,5-c]pyridine silylated with 0.5

ml of N,O-bis(trimethylsily)acetamide in 10 ml of acetonitrile. The reaction mixture was stirred for 3 hours at 25 °C and then added to a mixture of 0.5 ml of methanol and 5 ml of acetonitrile at 0 °C. The mixture was stirred at 0 °C for 30 minutes. The precipitated solids were collected by filtration to give a solid product. 10 Ml of water was added to the solid, and the mixture was neutralized with a saturated sodium bicarbonate solution and then concentrated. The residue was purified by chromatography over silica gel to give 50 mg of the title compound.

m.p. : 250 °C (decomp.)

IR (KBr, cm^{-1}) : 1760; 1616; 1590.

NMR (D_2O) : 8.34(1H,s); 8.1(1H,s); 7.35(1H,d); 7.00(1H,s); 5.9(1H,d); 5.15(1H,d); 4.60(2H,q); 3.80(2H,q); 3.35(2H,q); 3.15(3H,s).

EXAMPLE 17: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-METHOXYIMINOACETAMIDO]-3-[1-AMINO-2(3H)-OXO-IMIDAZO[4,5-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

To a suspension of 650 mg of 7-ß-[(z)-2-(aminothiazol-4-ly)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid in 15 ml of dry dichloromethane was added, in one portion, 7.8 ml of N-methyl-N-(trimethylsily)trifluoroacetamide at room temperature. The reaction mixture was stirred for 5 minutes at 25 °C under a nitrogen atmosphere. To the stirred solution were added by pipette 0.49 ml of iodotrimethylsilane at 0 °C, and the reaction mixture was then stirred at room temperature for 30 minutes. Thereafter, the mixture was evaporated under reduced pressure to remove the solvent and then give an oil. The oil was dissolved in a mixture of 10 ml of acetonitrile and 1 ml of tetrahydrofuran, and the resulting

solution was stirred for 5 minutes. The stirred solution was added, in one portion, to a solution of 165 mg of 1-amino-2(3H)-oxo-imidazo[4,5-c]pyridine silylated with 8.2 ml of N,O-bis(trimethylsilyl)acetamide in 10 ml of acetonitrile. The reaction mixture was stirred for 3 hours at 25 °C and then added to a mixture of 0.5 ml of methanol and 5 ml of acetonitrile at 0 °C. The mixture was stirred at 0 °C for 30 minutes. The precipitated solids were collected by filtration to give a solid product. 10 Ml of water was added to the solid and the mixture was neutralized with a saturated sodium bicarbonate solution and then concentrated. The residue was purified by chromatography over silica gel to give 120 mg of the title compound.

m.p. : 224 °C (decomp.)

IR (KBr, cm^{-1}) : 1785; 1653.

NMR (DMSO-d_6) : 9.6(1H,d, J=7.6Hz); 8.9(1H,s); 8.6(1H,d, J=6.6Hz); 7.5(1H,d, J=6.6Hz); 7.2(2H,b); 6.7(1H,m); 6.6(2H,bd); 5.8(1H,dd, J=7.8, 5.0Hz); 5.1(1H,d, J=5.0Hz); 5.4(2H,bd); 3.8(3H,s); 3.4(2H,m).

EXAMPLE 18: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-METHOXYIMINOACETAMIDO]-3-[1-(2-HYDROXYETHYL)-2(3H)-OXO-IMIDAZO[4,5-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

To a suspension of 700 mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid in 70 ml of dry dichloromethane was added, in one portion, 16 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide at room temperature. The reaction mixture was stirred for 5 minutes at 25 °C under a nitrogen atmosphere. To the stirred solution was added by pipette 0.5 ml of iodotrimethylsilane at 0 °C, and the reaction mixture was then stirred at room temperature for 30

minutes. Thereafter, the mixture was evaporated under reduced pressure to remove the solvent and then give an oil. The oil was dissolved in a mixture of 10 ml of acetonitrile and 1 ml of tetrahydrofuran, and the solution was stirred for 5 minutes. The stirred solution was added, in one portion, to a solution of 160 mg of 1-(2-hydroxyethyl)-2(3H)-oxo-imidazo[4,5-c]pyridine silylated with 8.3 ml of N,O-bis(trimethylsilyl)acetamide in 10 ml of acetonitrile. The reaction mixture was stirred for 3 hours at 25 °C and then added to a mixture of 0.6 ml of methanol and 6 ml of acetonitrile at 0°C. The mixture was stirred at 0 °C for 30 minutes. The precipitated solids were collected by filtration to give a solid product. 70 Ml of water was added to the solid, and the mixture was neutralized with a saturated sodium bicarbonate solution and then concentrated. The residue was purified by chromatography over silica gel to give 115 mg of the title compound.

m.p. : 250 °C

NMR : 9.5(1H,d); 8.7(1H,s); 8.5(1H,d); 7.4(1H,d); 7.1(1H,s); 6.7(1H,s); 5.6(1H,dd); 5.1(1H,d); 3.75(3H,s); 3.0-3.6(4H,m)

EXAMPLE 19: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-METHOXYIMINOACETAMIDO]-3-[2(1H,3H)-OXO-IMIDAZO[4,5-b]-PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

To a suspension of 500mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid in 10 ml of dry dichloromethane was added, in one portion, 0.74 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide at room temperature. The reaction mixture was stirred for 5 minutes at 25 °C under a nitrogen atmosphere. To the stirred solution was added by pipette 0.39 ml of iodotrimethylsilane at 0 °C, and the reaction mixture was then stirred at room temperature for

30 minutes. Thereafter, the solvent was evaporated off under reduced pressure to give an oil. The oil was dissolved in a mixture of 15 ml of acetonitrile and 0.5 ml of tetrahydrofuran, and the solution was stirred for 5 minutes. The stirred solution was added, in one portion, to a solution of 150 mg of 2(1H,3H)-oxo-imidazo[4.5-b]-pyridine silylated with 1.1 ml of N,O-bis(trimethylsily)-acetamide in 3 ml of acetonitrile. The reaction mixture was stirred for 3 hours at 25 °C and then added to a mixture of 0.5 ml of methanol and 5 ml of acetonitrile at 0°C. The mixture was stirred at 0°C for 30 minutes. The precipitated solids were collected by filtration to give a solid product. 10 Ml of water was added to the solid, and the mixture was neutralized with a saturated sodium bicarbonate solution and then concentrated. The residue was purified by chromatography over silica gel to give 110 mg of the title compound.

m.p. : 256°C (decomp.)

IR (KBr, cm^{-1}) : 1668; 1769.

NMR (D_2O) : 3.3(2H,q); 3.95(3H,s); 4.66(2H,q); 5.2(1H,d, J=4.9Hz); 6.96(1H,m); 6.9(1H,s); 7.4(1H,d, J=7.4Hz); 7.75(1H,d, J=5.4Hz).

EXAMPLE 20: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-FLUOROMETHOXYIMINOACETAMIDO]-3-[2(1H,3H)-OXO-IMIDAZO-[4,5-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

To a suspension of 700 mg of 7-ß-[(Z)-2-(2-amino-thiazol-4-yl)-2-fluoromethoxyiminoacetamido]-3-acetoxy-methyl-3-cephem-4-carboxylic acid in 15 ml of dry dichloro-methane was added, in one portion, 1.0 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide at room temperature. The reaction mixture was stirred for 5 minutes at 25 °C under a nitrogen atmosphere. To the stirred solution was added by pipette 0.44 ml of iodotrimethylsilane at 0 °C,

and the reaction mixture was then stirred at room temperature for 30 minutes. Thereafter, the mixture was evaporated under reduced pressure to remove the solvent and then give an oil. The oil was dissolved in a mixture of 10 ml of acetonitrile and 1.0 ml of tetrahydrofuran, and the solution was stirred for 5 minutes. The stirred solution was added in one portion to a solution of 178 mg of 2(1H,3H)-oxo-imidazo[4,5-c]pyridine silylated with 0.88 ml of N,O-bis(trimethylsily)acetamide in 10 ml of acetonitrile. The reaction mixture was stirred for 3 hours at 25 °C and then added to a mixture of 1.0 ml of methanol and 2 ml of acetonitrile at 0 °C. The mixture was stirred at 0 °C for 30 minutes. The precipitated solids were collected by filtration to give a solid product. 10 Ml of water was added to the solid, and the mixture was neutralized with a saturated sodium bicarbonate solution and then concentrated. The residue was purified by chromatography over silica gel to give 110 mg of the title compound.

m.p. : 210°C (decomp.)

IR (KBr, cm^{-1}) : 1763; 1653; 1616.

NMR (D_2O) : 3.18(1H,d); 3.58(1H,d); 4.41(1H,s); 4.99 (1H,s); 5.30(1H,d, J=4.80Hz); 5.81(2H,d, J=55.07Hz); 5.88(1H,d, J=4.80Hz); 7.15 (1H,s); 7.46(1H,d, J=6.9Hz); 8.35(1H,d, J=6.9Hz); 8.46(1H,s).

EXAMPLE 21: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-FLUOROMETHOXYIMINOACETAMIDO]-3-[1-METHYL-2(3H)-OXO-IMIDAZO[4,5-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

To a suspension of 300 mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-fluoroemthoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid in 5 ml of dry dichloromethane was added, in one portion, 0.5 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide at room temperature.

The reaction mixture was stirred for 5 minutes at 25 °C under a nitrogen atmosphere. To the stirred solution was added by pipette 0.3 ml of iodotrimethylsilane at 0 °C, and the reaction mixture was then stirred at room temperature for 30 minutes. Thereafter, the solvent was evaporated off under reduced pressure to give an oil. The oil was dissolved in a mixture of 10 ml of acetonitrile and 0.5 ml of tetrahydrofuran, and the resultant solution was stirred for 5 minutes. The stirred solution was then added, in one portion, to a solution of 85 mg of 1-methyl-2(3H)-oxo-imidazo[4,5-c]pyridine silylated with 0.71 ml of N,O-bis-(trimethylsilyl)acetamide in 3 ml of acetonitrile. The reaction mixture was stirred for 3 hours at 25 °C and then added to a mixture of 0.3 ml of methanol and 2 ml of aceto-nitrile at 0 °C. The mixture was stirred at 0°C for 30 minutes. The precipitated solids were collected by filtration to give a solid product. 10 Ml of water was added to the solid, and the mixture was neutralized with a saturated sodium bicarbonate solution and then concent-rated. The residue was purified by chromatography over silica gel to give 60 mg of the title compound.

m.p. : 242 °C (decomp.)

IR (KBr, cm^{-1}) : 1533; 1616; 1751.

NMR (D_2O) : 8.35(1H,s); 8.2(1H,s); 7.35(1H,d); 7.06 (1H,s); 5.78(2H,d, J=55.4Hz); 5.85(1H,d); 5.3(1H,d); 4.55 (2H,q); 3.35(2H,q).

EXAMPLE 22: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-FLUOROMETHOXYIMINOACETAMIDO]-3-[1-AMINO-2(3H)-OXO-IMIDAZO[4,5-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

To a suspension of 500 mg of 7-ß-[(Z)-2-(2-amino-thiazol-4-yl)-2-fluoromethoxyiminoacetamido]-3-acetoxy-methyl-3-cephem-4-carboxylic acid in 10 ml of dry dichloro-methane was added, in one portion, 0.65 ml of N-methyl-N-

(trimethylsilyl)trifluoroacetamide at room temperature. The reaction mixture was stirred for 5 minutes at 25 °C under a nitrogen atmosphere. To the stirred solution was added by pipette 0.45 ml of iodotrimethylsilane at 0 °C, and the reaction mixture was then stirred at room temperature for 30 minutes. Thereafter, the solvent was evaporated off under reduced pressure to give an oil. The oil was dissolved in a mixture of 10 ml of acetonitrile and 1.0 ml of tetrahydrofuran, and the solution was stirred for 5 minutes. The stirred solution was added, in one portion, to a solution of 160 mg of 1-amino-2(3H)-oxo-imidazo[4,5-c]pyridine silylated with 0.71 ml of N,O-bis(trimethylsily)acetamide in 10 ml of acetonitrile. The reaction mixture was stirred for 3 hours at 25 °C and then added to a mixture of 1.0 ml of methanol and 2 ml of acetonitrile at 0 °C. The mixture was stirred at 0 °C for 30 minutes. The precipitated solids were collected by filtration to give a solid product. 10 Ml of water was added to the solid and the mixture was neutralized with a saturated sodium bicarbonate solution and then concentrated. The residue was purified by chromatography over silica gel to give 105 mg of the title compound.

m.p. : 215 °C (decomp.)

IR (KBr, cm^{-1}) : 1773; 1654.

NMR (DMSO-d_6) : 9.75(1H,d); 8.65(1H,s); 8.55(1H,d, J=6.7Hz); 7.65(1H,d, J=6.7Hz); 7.2 (2H,m); 6.9(1H,s); 5.8 (1H,m); 5.6 (2H,d, J=55.0Hz); 5.4(2H,bd); 5.15 (1H,d); 3.4(2H,m).

EXAMPLE 23: SYNTHESIS OF 7-ß-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-FLUOROMETHOXYIMINOACETAMIDO]-3-[2(1H,3H)-OXO-IMIDAZO-[4,5-b]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

To a suspension of 300 mg of 7-ß-[(Z)-2-(2-aminothia-

zol-4-yl)-2-fluoromethoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid in 10 ml of dry dichloromethane was added, in one portion, 0.55 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide at room temperature. The reaction mixture was stirred for 5 minutes at 25 °C under a nitrogen atmosphere. To the stirred solution was added by pipette 0.33 ml of iodotrimethylsilane at 0 °C, and the reaction mixture was then stirred at room temperature for 30 minutes. Thereafter, the solvent was evaporated off under reduced pressure to give an oil. The oil was dissolved in a mixture of 15 ml of acetonitrile and 0.3 ml of tetrahydrofuran, and the solution was stirred for 5 minutes. The stirred solution was added, in one portion, to a solution of 100 mg of 2(1H,3H)-oxo-imidazo[4,5-b]-pyridine silylated with 0.90 ml of N,O-bis(trimethylsilyl)-acetamide in 3 ml of acetonitrile. The reaction mixture was stirred for 3 hours at 25 °C and then added to a mixture of 0.5 ml of methanol and 2 ml of acetonitrile at 0 °C. The mixture was stirred at 0 °C for 30 minutes. The precipitated solids were collected by filtration to give a solid product. 10 Ml of water was added to the solid, and the mixture was neutralized with a saturated sodium bicarbonate solution and then concentrated. The residue was purified by chromatography over silica gel to give 20 mg of the title compound.

m.p. : 231 °C (decomp.)

IR (KBr, cm^{-1}) : 1616; 1668; 1767.

NMR (D_2O) : 3.40(2H,q); 4.60(2H,q); 5.25(1H,d, J=4.80Hz); 5.85(2H,d, J=55.0Hz); 5.95 (1H,d, J=4.80Hz); 7.10(1H,m); 7.15(1H,s); 7.50(1H,d); 7.75(1H,d).

EXAMPLE 24: SYNTHESIS OF 7-β-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-CARBOXYMETHOXYIMINOACETAMIDO]-3-[2(1H,3H)-OXO-IMIDAZO-[4,5-c]PYRIDINIUMMETHYL]-3-CEPHEM-4-CARBOXYLATE

To a suspension of 700 mg of 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-carboxymethoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid in 15 ml of dry dichloromethane was added, in one portion, 0.95 ml of N-methyl-N-(trimethylsilyl)trifluoroacetamide at room temperature. The reaction mixture was stirred for 5 minutes at 25 °C under a nitrogen atmosphere. To the stirred solution was added by pipette 0.40 ml of iodotrimethylsilane at 0 °C, and the reaction mixture was then stirred at room temperature for 30 minutes. Thereafter, the solvent was evaported off under reduced pressure to give an oil. The oil was dissolved in a mixture of 10 ml of acetonitrile and 1.0 ml of tetrahydrofuran, and the solution was stirred for 5 minutes. The stirred solution was added, in one portin, to a solution of 180 mg of 2(1H,3H)-oxo-imidazo[4,5-c]pyridine silylated with 0.80 ml of N,O-bis(trimethylsilyl)acetamide in 10 ml of acetonitrile. The reaction mixture was stirred for 3 hours at 25 °C and then added to a mixture of 1.0 ml of methanol and 2 ml of acetonitrile at 0 °C. The mixture was stirred at 0 °C for 30 minutes. The precipitated solids were collected by filtration to give a solid product. 10 Ml of water was added to the solid, and the mixture was neutralized with a saturated sodium bicarbonate solution and then concentrated. The residue was purified by chromatography over silica gel to give 80 mg of the title compound.

m.p. : 228 °C (decomp.)

IR (KBr, cm^{-1}) : 1761; 1653; 1616.

NMR (DMSO-d_6) : 3.35(1H,q); 4.50(2H,s); 4.75(2H,q); 5.25(1H,d, J=4.7Hz); 5.85(1H,d, J=4.7Hz); 6.95(1H,s); 7.45 (1H,d, J=6.7Hz); 8.35(1H,d, J=6.7Hz); 8.60 (1H,s).

The title compounds illustrated in the above Examples are summarized in Table 1 below.

Table 1

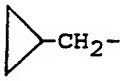
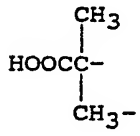
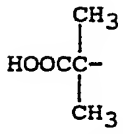
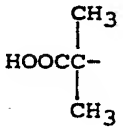
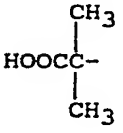
Example No.	n	R_1	R_2	R_3	Fused position
Ex. 1	2	CH_3-	H	H	3,4-fused
Ex. 2	2	CH_3CH_2-	H	H	3,4-fused
Ex. 3	2	HC C-CH_2-	H	H	3,4-fused
Ex. 4	2		H	H	3,4-fused
Ex. 5	2	$HOOCCH_2$-	H	H	3,4-fused
Ex. 6	2		H	H	3,4-fused
Ex. 7	2	CH_3-	CH_3-	H	3,4-fused
Ex. 8	2	CH_3-	CH_3CH_2-	H	3,4-fused
Ex. 9	2	CH_3-		H	3,4-fused
Ex.10	2		CH_3-	H	3,4-fused
Ex.11	2		CH_3CH_2-	H	3,4-fused
Ex.12	2			H	3,4-fused
Ex.13	2	CH_3-	H	CH_3-	3,4-fused
Ex.14	2	FCH_2-	H	H	3,4-fused

Table 1 (Continued)

Example No.	n	R_1	R_2	R_3	Fused position
Ex.15	1	CH_3-	H	H	3,4-fused
Ex.16	1	CH_3-	CH_3-	H	3,4-fused
Ex.17	1	CH_3-	H_2N-	H	3,4-fused
Ex.18	1	CH_3-	$HOCH_2CH_2$-	H	3,4-fused
Ex.19	1	CH_3-	H	H	2,3-fused
Ex.20	1	FCH_2-	H	H	3,4-fused
Ex.21	1	FCH_2-	CH_3-	H	3,4-fused
Ex.22	1	FCH_2-	H_2N-	H	3,4-fused
Ex.23	1	FCH_2-	H	H	2,3-fused
Ex.24	1	$HOOCCH_2$-	H	H	3,4-fused

INDUSTRIAL APPLICABILITY

The advantageous effects accruing from and the industrial applicability of the present invention are illustrated by means of the following experimental examples.

EXPERIMENTAL EXAMPLE 1: *in vitro* ACTIVITY

The *in vitro* antibacterial activities of several representative compounds of the present invention against various gram-positive and gram-negative microorganisms were evaluated by the following two-fold dilution method. As reference compounds, cefotaxime (CTX) and ceftazidime (CAZ) were employed.

Two-fold serial dilutions of the compounds of Examples 1, 2, 3, 5, 6, 7, 9 and 10, and the reference compounds were prepared. 1.5 Ml of each dilution and subsequently 13.5 ml of Mueller-Hinton agar were added into a test tube and then mixed together. After thoroughly mixing, the mixture was poured into a sterilized Petri dish and coagulated. Each test microorganism-diluted suspension (about 10^4 cfu/spot) was inoculated to the Mueller Hinton agar with an inoculator. After incubation at 37°C for 18 hours, the minimum inhibitory concentrations (MICs: μg/ml) of the test and the reference compounds were measured. The results are shown in Table 2 below.

Table 2

Strain		Compound of Ex. 1	Ex. 2	Ex. 3	Ex. 5	Ex. 6	Ex. 7	Ex. 9	Ex.10	CTX	CAZ
S.pyogenes	A8668	0.013	0.013	0.013	0.196	0.782	0.025	0.049	0.782	0.013	0.196
S.aureus	A29213	1.563	1.563	0.782	25	12.5	1.563	3.125	12.5	0.782	12.5
S.epidermidis	A12228	0.391	0.391	0.391	1.563	3.125	0.391	1.563	3.125	0.391	6.25
E.coli	A10536	0.007	0.013	0.013	0.002	0.007	0.007	0.025	0.007	0.013	0.098
P.mirabilis	A25933	0.013	0.025	0.013	<0.002	0.007	0.007	0.049	0.013	0.013	0.049
S.marcescens	A27117	0.025	0.049	0.049	0.004	0.007	0.007	0.782	0.013	0.049	0.391
P.aeruginosa	A10145	3.125	6.25	3.125	1.563	1.563	12.5	25	6.25	25	3.125

EXPERIMENTAL EXAMPLE 2: in vitro ACTIVITY

In order to further illustrate the in vitro antibacterial activities of other representative compounds of the present invention, the minimal inhibitory concentrations (MIC) thereof against various gram-positive and gram-negative microorganisms were determined, and compared with those of cefotaxime (CTX) and ceftazidime (CAZ). The in vitro antibacterial activities were determined by the two-fold dilution method similar to that described in Experimental Example 1.

The two-fold serial dilutions of the test compounds and reference compounds were made and dispersed in Muller-Hinton agar medium. Then, 2 μl of standard test strain which had 10^4 cfu/spot was inoculated on the medium, and was incubated at 37 °C for 20 hours. After the incubation, MICs (μg/ml) of the test and reference compounds were measured. The results are shown in Table 3 below.

Table 3

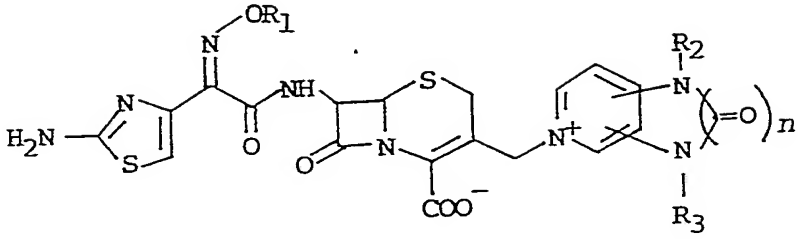
Strain		Compound of Ex.15	Ex.20	Ex.24	Ex.16	Ex.21	Ex.17	Ex.22
S.pyogenes	A8668	0.007	0.007	0.098	0.013	0.007	0.007	0.013
S.pyogenes	C4003	0.007	0.007	0.196	0.013	0.007	0.013	0.025
S.aureus	A29213	1.563	0.782	12.5	1.563	1.563	1.563	1.563
S.aureus	C4036	0.782	0.782	12.5	1.563	0.782	1.563	1.563
MRSA	C1060	25	25	50	100	25	100	50
S.epidermidis	A12228	0.391	0.391	6.25	0.391	0.391	0.391	0.782
E.coli	A10536	0.004	≤0.002	0.025	0.007	0.004	0.007	0.007
E.coli	A25922	0.013	0.007	0.049	0.013	0.013	0.013	0.025
E.coli	C4052	0.007	0.004	0.025	0.007	0.007	0.007	0.013
E.cloacae	C4008	0.004	≤0.002	0.013	0.004	0.004	0.004	0.007
E.cloacae	C4009	0.013	0.007	0.013	0.013	0.007	0.013	0.013
K.oxytoca	C4022	1.563	0.782	0.782	0.782	0.391	1.563	1.563
K.pneumoniae	A10031	0.007	0.004	0.025	0.007	0.004	0.007	0.007
K.pneumoniae	C4021	0.007	0.004	0.025	0.007	0.004	0.007	0.013
P.mirabilis	A25933	0.013	0.004	0.007	0.013	0.007	0.013	0.013
P.rettgeri	A9919	0.004	0.004	0.007	0.004	0.004	0.007	0.013
S.typhimurium	C4045	0.013	0.007	0.049	0.013	0.007	0.013	0.013
S.marcescens	A27117	0.013	0.007	0.025	0.013	0.007	0.013	0.013
P.aeruginosa	A10145	1.563	1.563	3.125	3.125	1.563	3.125	3.125
P.aeruginosa	C4028	0.004	≤0.002	0.007	0.007	0.004	0.007	0.007
P.aeruginosa	A27853	0.782	0.782	1.563	1.563	0.782	1.563	1.563

Table 3 (Continued)

Strain		Compound of Ex.19	Compound of Ex.23	CTX	CAZ
S.pyogenes	A8668	0.007	0.013	0.007	0.098
S.pyogenes	C4003	0.007	0.013	0.007	0.196
S.aureus	A29213	0.782	0.782	0.782	6.25
S.aureus	C4036	0.782	0.782	0.782	6.25
MRSA	C1060	25	25	100	100
S.epidermidis	A12228	0.391	0.391	0.391	3.125
E.coli	A10536	0.025	0.025	0.013	0.049
E.coli	A25922	0.098	0.098	0.049	0.196
E.coli	C4052	0.049	0.025	0.013	0.098
E.cloacae	C4008	0.013	0.013	0.007	0.025
E.cloacae	C4009	0.098	0.049	0.049	0.098
K.oxytoca	C4022	50	12.5	0.782	0.782
K.pneumoniae	A10031	0.013	0.007	0.004	0.098
K.pneumoniae	C4021	0.013	0.013	0.004	0.049
P.mirabilis	A25933	0.007	0.007	0.013	0.049
P.rettgeri	A9919	0.004	0.007	0.004	0.025
S.typhimurium	C4045	0.049	0.025	0.013	0.196
S.marcescens	A27117	0.049	0.049	0.049	0.098
P.aeruginosa	A10145	25	12.5	25	3.125
P.aeruginosa	C4028	0.004	0.007	$\leq$0.002	0.013
P.aeruginosa	A27853	12.5	6.25	12.5	1.563

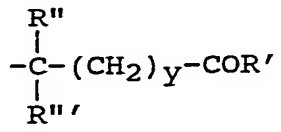
CLAIMS

1. A compound of the formula:



(I)

wherein, R_1 is hydrogen, or a lower alkyl, C_3-C_4 alkenyl, C_3-C_4 alkynyl or cycloalkylalkyl group, a fluoro-substituted lower alkyl group represented by the formula: $-(CH_2)_xF$ in which x is an integer of 1 to 3, or a carboxy-substituted alkyl group represented by the formula:



wherein R′ is a hydroxy, amino or C_1-C_4 alkoxy group; R" and R"′, which may be the same or different, represent hydrogen or a C_1-C_3 alkyl group, or R" and R"′ together with the carbon atom to which they are attached may form a C_3-C_7 carbocyclic ring; and y is an integer of 0 to 3;

R_2 and R_3, which may be the same or different, represent hydrogen, or a lower alkyl, amino, carboxy-substituted lower alkyl, hydroxy-substituted lower alkyl or C_3-C_7 cycloalkyl group;

n is an integer of 1 or 2; and

the 2-oxo-heterocyclic moiety is fused with the pyridine ring to form a 2,3- or 3,4-fused ring substituent at 3-position of the cephem nucleus; or a

pharmaceutically acceptable salt, physiologically hydrolyzable ester or solvate thereof.

2. The compound of Claim 1, wherein R_1 is a methyl, ethyl, cyclopropyl, fluoromethyl, 2-carboxyprop-2-yl or carboxymethyl group; R_2 is hydrogen, or a methyl, ethyl, cyclopropyl, amino or hydroxyethyl group; and R_3 is hydrogen or a methyl group.

3. The compound of Claim 1, which is 7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[2,3-(1H,4H)-dioxo-pyrazino[5,6-c]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-ethoxyiminoacetamido]-3-[2,3(1H,4H)-dioxo-pyrazino[5,6-c]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-aminothiazol-4-yl)-2-propynyloxyiminoacetamido]-3-[2,3(1H,4H)-dioxo-pyrazino[5,6-c]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-cyclopropylmethoxyiminoacetamido]-3-[2,3(1H,4H)-dioxo-pyrazino[5,6-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-carboxymethoxyimino acetamido]-3-[2,3(1H,4H)-dioxo-pyrazino[5,6-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-yl)oxyiminoacetamido]-3-[2,3(1H,4H)-dioxo-pyrazino-[5,6-c]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[1-methyl-2,3(4H)-dioxo-pyrazino[5,6-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[1-ethyl-2,3(4H)-dioxo-pyrazino[5,6-c]-pyridimiummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[1-cyclopropyl-2,3(4H)-dioxo-pyrazino[5,6-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-yl)oxyiminoacetamido]-3-[1-methyl-2,3(4H)-dioxo-pyrazino[5,6-c]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-yl)oxyiminoacetamido]-3-[1-ethyl-2,3(4H)-dioxo-pyrazino[5,6-c]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-yl)oxyiminoacetamido]-3-[1-cyclopropyl-2,3(4H)-dioxo-pyrazino[5,6-c]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl]-2-methoxyiminoacetamido]-3-[4-methyl-2,3(1H)-dioxo-pyrazino[5,6-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(aminothiazol-4-yl)-2-fluoromethoxyimino-acetamido]-3-[2,3(1H,4H)-dioxo-pyrazino[5,6-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[2(1H,3H)-oxo-imidazo[4,5-c]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[1-methyl-2(3H)-oxo-imidazo[4,5-c]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[1-amino-2(3H)-oxo-imidazo[4,5-c]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[1-(2-hydroxyethyl)-2(3H)-oxo-imidazo[4,5-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[2(1H,3H)-oxo-imidazo[4,5-b]pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-fluoromethoxyiminoacetamido]-3-[2(1H,3H)-oxo-imidazo[4,5-c]pyridiniummethyl]-3-cephem-4-carboxylate;

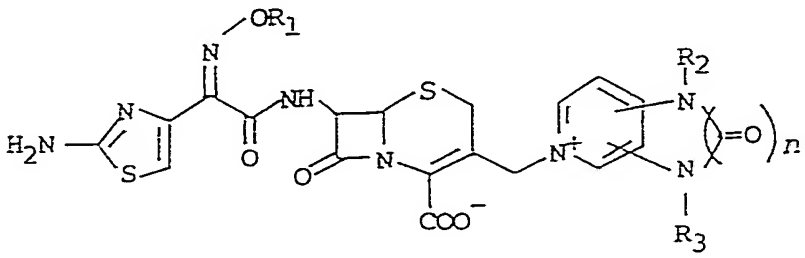
7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-fluoromethoxyiminoacetamido]-3-[1-methyl-2(3H)-oxo-imidazo[4,5-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-fluoromethoxyiminoacetamido]-3-[1-amino-2(3H)-oxo-imidazo[4,5-c]-pyridiniummethyl]-3-cephem-4-carboxylate;

7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-fluoromethoxyiminoacetamido]-3-[2(1H,3H)-oxo-imidazo[4,5-b]pyridiniummethyl]-3-cephem-4-carboxylate; or

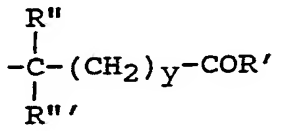
7-ß-[(Z)-2-(2-aminothiazol-4-yl)-2-carboxymethoxy-iminoacetamido]-3-[2(1H,3H)-oxo-imidazo[4,5-c]-pyridiniummethyl]-3-cephem-4-carboxylate.

4. A process for preparing a compound of the formula:



(I)

wherein R_1 is hydrogen, or a lower alkyl, C_3-C_4 alkenyl, C_3-C_4 alkynyl or cycloalkylalkyl group, a fluoro-substituted lower alkyl group represented by the formula: $-(CH_2)_xF$ in which x is an integer of 1 to 3, or a carboxy-substituted alkyl group represented by the formula:



wherein R′ is a hydroxy, amino or C_1-C_4 alkoxy group; R" and R"′, which may be the same or different, represent hydrogen or a C_1-C_3 alkyl group, or R" and R"′ together with the carbon atom to which they are attached may form a C_3-C_7 carbocyclic ring; and y is an integer of 0 to 3;

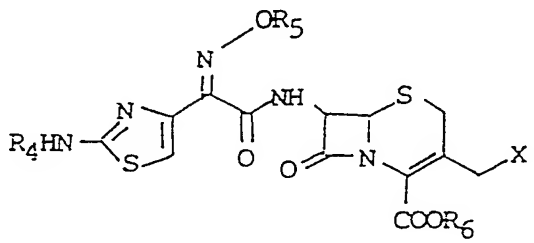
R_2 and R_3, which may be the same or different, represent hydrogen, or a lower alkyl, amino, carboxy-substituted lower alkyl, hydroxy-substituted lower alkyl or C_3-C_7 cycloalkyl group;

n is an integer of 1 or 2; and

the 2-oxo-heterocyclic moiety is fused with the pyridine ring to form a 2,3- or 3,4-fused ring substituent at 3-position of the cephem nucleus; or a

pharmaceutically acceptable salt, physiologically hydrolyzable ester or solvate thereof, which comprises the steps of:

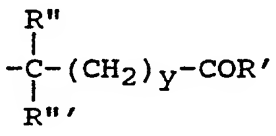
reacting a compound of the formula:



(II)

wherein R_4 is an amino protecting group;

R_5 is hydrogen or a lower alkyl, C_3-C_4 alkenyl, C_3-C_4 alkynyl or cycloalkylalkyl group, a fluoro-substituted lower alkyl group represented by the formula: $-(CH_2)xF$ in which x is an integer of 1 to 3, or a carboxy-substituted alkyl group represented by the formula:

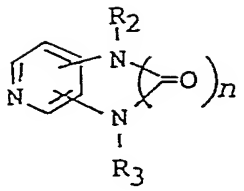


wherein R′ is a hydroxy, amino or C_1-C_4 alkoxy group; R" and R"′ may be the same or different and represent hydrogen or a C_1-C_3 alkyl group, or R" and R"′ together with the carbon atom to which they are attached may form a C_3-C_7 carbocyclic ring; and y is an integer of 0 to 3;

R_6 is a carboxyl protecting group; and

X is a leaving group;

with a compound of the formula:



(III)

wherein R_2, R_3 and n have the same meaning as defined above and the 2-oxo-heterocyclic moiety is fused with the pyridine ring to form a 2,3- or 3,4-fused ring in the presence of an organic solvent; and

then, if necessary, removing the amino protecting group and/or the carboxyl protecting group.

5. The process of Claim 4, wherein the organic solvent is selected from the group consisting of a nitrile solvent such as acetonitrile and propionitrile; an alkyl halide solvent such as chloroform, carbon tetrachloride and dichloromethane; an ether solvent such as tetrahydrofuran and dioxane; an amide solvent such as N,N-dimethylformamide; an ester solvent such as ethylacetate and methylacetate; a ketone solvent such as acetone, methyl ethyl ketone and methyl isobutyl ketone; a sulfoxide solvent such as dimethylsulfoxide; and an aromatic hydrocarbon solvent such as benzene and toluene.

6. The process of Claim 4, wherein the compound of the formula (II) is used in an amount of from 1 to 2 equivalents based on 1 equivalent of the compound of the formula (III).

7. The process of Claim 4, wherein a compound of the formula (II) in which X is an acetoxy group is first silylated with a silylating agent to protect the carboxy group at 4-position and the amino group of the substituent at 7-position, and the resulting silylated compound is then reacted with trimethyl silyliodide to form a compound of the formula (II) in which X is iodine, followed by reacting

with a silylated fused pyridine of the formula (III).

8. The process of Claim 4, wherein the silylating agent is selected from the group consisting of mono- or bis-trimethylsilylacetamide, N-methyl-N-(trimethylsilyl)acetamide, N,O-bis(trimethylsilyl)trifluoroacetamide, N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA), and hexamethyldisilazane (HMDS).

9. The process of Claim 4, wherein the reaction is carried out in the presence of one or more stabilizing agents.

10. The process of Claim 9, wherein the stabilizing agent is selected from the group consisting of sodium iodide, potassium iodide, sodium bromide, potassium bromide, and potassium thiocyanate.

11. A pharmaceutical composition which comprises a therapeutically effective amount of one or more of the cephalosporin compounds of the formula (I) according any of Claims 1 to 3, or a pharmaceutically acceptable salt, physiologically hydrolyzable ester or slovate thereof, in association with a pharmaceutically acceptable carrier, excipient, or other additives therefor.

12. A compound of the formula (I) according to any of Claims 1 to 3 for use as antibiotics.

13. Use of a compound of the formula (I) as defined in any of Claims 1 to 3 for manufacturing a medicament for antibiotic use.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 92/00016

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl.[5]: C 07 D 519/00, 501/46; A 61 K 31/545

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl.[5]: C 07 D 519/00, 501/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

AT

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4 748 172 (A. S. KATNER) 31 May 1988 (31.05.88), see claims 1-3; column 2, line 5 to column 3, line 50; column 8, line 1 to column 9, line 36.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 July 1992 (01.07.92)	13 July 1992 (13.07.92)

Name and mailing address of the ISA/	Authorized officer
AUSTRIAN PATENT OFFICE Kohlmarkt 8-10 A-1014 VIENNA	Mazzucco e.h.
Facsimile No. 0222/53424/535	Telephone No. 0222/5337058/733

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR 92/00016

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US A 4748172	31-05-88	AU A1 34189/84	26-04-85
		AU B2 574107	30-06-88
		CA A1 1225390	11-08-87
		DK AO 4891/84	12-10-84
		DK A 4891/84	18-04-85
		EP A2 138552	24-04-85
		EP A3 138552	19-03-86
		ES A1 536728	16-11-85
		ES A5 536728	16-12-85
		ES A1 8602816	16-03-86
		FI AO 844000	11-10-84
		FI A 844000	18-04-85
		GB AO 8425453	14-11-84
		GB A1 2148289	30-05-85
		GB AO 8627171	10-12-86
		GB A1 2181136	15-04-87
		GB B2 2148289	23-09-87
		GB B2 2181136	25-05-88
		HU A2 35687	29-07-85
		HU B 195512	30-05-88
		IL AO 73230	31-01-85
		JP A2 60105685	11-06-85
		NZ A 209833	29-04-88
		PH A 21040	03-07-87
		PT A 79357	01-11-84
		PT B 79357	20-11-86
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		ZA A 8407926	28-05-86
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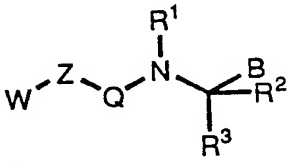

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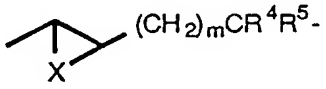
(a) 

(57) Abstract

Compounds of general formula (I), wherein W represents pyrid-3-yl, benzimidazol-1-yl, imidazo(4,5-c)pyridin-1-yl, imidazo(4,5-c)pyridin-3-yl and imidazo(4,5-c)pyridin-5-yl optionally substituted by alkyl; Z represents: a) a divalent alkanediyl, alkenediyl or alkynediyl group, b) a $-(CH_2)_qU(CH_2)_r-$ group, optionally substituted, q is an integer from 0-3, r is an integer from 0-3 and U is -O-, -S- or a furandiyl, tetrahydrofurandiyl, thiophenediyl, tetrahydrothiophenediyl, thiazolediyl, tetrahydrothiazolediyl, piperazinediyl, piperidinediyl, cyclopentanediyl, cyclohexanediyl, cycloheptenediyl or benzenediyl group; or c) a (α) group wherein m is an integer from 0-3, X is -O-, -S- or $-CH_2-$ and each of R^4 and R^5 is independently hydrogen or alkyl; Q represents a carbonyl, thiocarbonyl or sulphonyl group or a bond; B represents: a) a $-VR^8$ group wherein V is $-C(=O)-$, $-C(=O)O-$, $-CH_2O-$, $-CH_2OC(=O)-$, $-C(=S)-$, $-CH_2OC(=O)NH-$, $-C(=S)O-$, $-CH_2S-$, $-C(=O)NHSO_2-$ or $-SO_2NHC(=O)-$; b) a $-CH_2NR^9R^{10}$ group or a $-CONR^9R^{10}$ group; c) a group Y where Y is a 5- or 6-membered heterocyclic ring; d) a group $-CH_2-Y$ or $C(=O)NHY$; are antagonists of platelet activating factor (PAF).

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AMINO ACID DERIVATIVES AS PAF-RECEPTOR ANTAGONISTS

This invention relates primarily to novel substituted amino acid derivatives that possess pharmaceutical activity as antagonists of PAF.

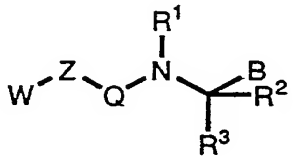
Platelet activating factor (PAF) is a bioactive phospholipid which has been identified as 1-*O*-hexadecyl/octadecyl-2-acetyl-*sn*-glyceryl-3-phosphoryl choline. PAF is released directly from cell membranes and mediates a range of potent and specific effects on target cells resulting in a variety of physiological responses which include hypotension, thrombocytopenia, bronchoconstriction, circulatory shock, and increased vascular permeability (oedema/erythema). It is known that these physiological effects occur in many inflammatory and allergic diseases and PAF has been found to be involved in a number of such disorders including asthma, endotoxin shock, adult respiratory distress syndrome, glomerulonephritis, immune regulation, transplant rejection, gastric ulceration, psoriasis, and cerebral, myocardial and renal ischemia. Thus the compounds of the invention, by virtue of their ability to antagonise the actions of PAF, should be of value in the treatment of any of the above conditions and any other conditions in which PAF is implicated (e.g. embryo implantation).

Compounds that have been disclosed as possessing activity as PAF antagonists include compounds which are structurally related to the PAF molecule such as glycerol derivatives (EP-A-0238202), and heterocyclic compounds such as 5-oxy derivatives of tetrahydrofuran (US-4,888,337) and 2,5-diaryl tetrahydrofurans (EP-A-0144804). Recently a more potent 2,5-diaryl tetrahydrofuran derivative, (*trans*)-2-(3-methoxy-5-methylsulphonyl-4-propoxyphenyl)-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran (L-659,989) has been disclosed (EP-A-0199324). In our International patent application no. WO 91/17157 we disclose a series of γ-butyrolactol derivatives as PAF antagonists. The compounds of WO 91/17157 differ from the 5-oxy derivatives of tetrahydofuran described in US-4,888,337 and from the 2,5-diaryl tetrahydrofurans such as L-659,989, in that they feature an appended heterocycle with an unsubstituted sp^2 nitrogen atom. There are a number of other PAF antagonists, in addition to those of WO 91/17157, for which the presence of a heterocyclic sp^2 nitrogen atom appears to be an important requirement for activity (Whittaker, M., Curr. Opin. Thera. Patents 2(5), 583-623 (1992)).

For the compounds of the present invention the presence of a heterocyclic group possessing an unsubstituted sp^2 nitrogen atom is also a requirement for PAF antagonist activity. However, the compounds of the present invention differ from the other PAF antagonists refered to above in that they are amino acid derivatives.

The present invention provides novel and useful substituted amino acid derivatives and their pharmaceutically acceptable acid addition salts, and pharmaceutical uses thereof as PAF antagonists.

According to a first aspect of the invention there is provided a compound of general formula I:



I

wherein:

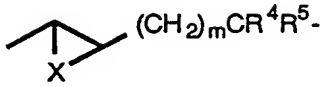
W represents pyrid-3-yl, benzimidazol-1-yl, imidazo[4,5-c]pyridin-1-yl, imidazo[4,5-c]pyridin-3-yl and imidazo[4,5-c]pyridin-5-yl optionally substituted with one or more -C_1-C_6 alkyl substituents;

Z represents:

a) a divalent alkanediyl, alkenediyl or alkynediyl group from 2 to 12 carbon atoms which may be a straight or branched-chain provided that, when Z represents a branched chain at least two carbon atoms separate W from the group Q, wherein the said group is either unsubstituted or substituted by one or more substituents selected from hydroxy, -OC_1-C_6 alkyl, -SC_1-C_6 alkyl and halo; or

b) a -$(CH_2)_qU(CH_2)_r$- group, optionally substituted by one or more substituents selected from hydroxy, -OC_1-C_6 alkyl, halo and nitrile, wherein q is an integer from 0-3, r is an integer from 0-3 and U is -O-, -S- or a furandiyl, tetrahydrofurandiyl, thiophenediyl, tetrahydrothiophenediyl, thiazolediyl, tetrahydrothiazolediyl, piperazinediyl, piperidinediyl, cyclopentanediyl, cyclohexanediyl, cycloheptenediyl or benzenediyl group (provided that, when U is a 1,4-benzenediyl group q is not an integer of 1); or

c) a

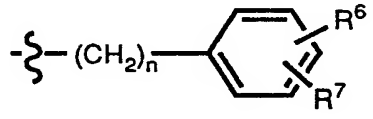


group wherein m is an integer from 0-3, X is -O-, -S- or $-CH_2-$ and each of R^4 and R^5 is independently hydrogen or $-C_1-C_6$ alkyl;

Q represents a carbonyl, thiocarbonyl or sulphonyl group or a bond

R^1 represents hydrogen, $-C_1-C_6$ alkyl, $-C_2-C_6$ alkenyl, $-C_2-C_6$ alkynyl, $-COC_1-C_6$ alkyl, $-CO_2C_1-C_6$ alkyl, $-(CO_2C_1-C_6$ alkyl)phenyl, $-(C_1-C_6$ alkyl)$CO_2C_1-C_6$ alkyl, $-(C_1-C_6$ alkyl)phenyl, $-C_3-C_8$ cycloalkyl, $-C_4-C_8$ cycloalkenyl or phenyl optionally substituted by one or more substituents selected from $-C_1-C_6$ alkyl, $-OC_1-C_6$ alkyl, halogen, $-CF_3$ and -CN;

R^2 represents hydrogen, halogen, $-C_1-C_6$ alkyl optionally substituted by one or more halogen atoms, $-C_2-C_6$ alkenyl, $-C_2-C_6$ alkynyl, $-(C_1-C_6$ alkyl)$CO_2C_1-C_6$ alkyl, $-(C_1-C_6$ alkyl)SC_1-C_6 alkyl, $-(C_1-C_6$ alkyl)OC_1-C_6 alkyl, $-(C_1-C_6$ alkyl)$N(C_1-C_6$ alkyl)$_2$, $-C_3-C_8$ cycloalkyl, $-C_4-C_8$ cycloalkenyl, $-(C_1-C_6$ alkyl)C_3-C_8 cycloalkyl, $-(C_1-C_6$ alkyl)C_4-C_8 cycloalkenyl, $-(C_1-C_6$ alkyl)OC_3-C_8 cycloalkyl, $-(C_1-C_6$ alkyl)OC_4-C_8 cycloalkenyl, $-(C_1-C_6$ alkyl)SC_3-C_8 cycloalkyl, $-(C_1-C_6$ alkyl)SC_4-C_8 cycloalkenyl, a side chain of a naturally occurring amino acid, a group -D or $-(C_1-C_6$ alkyl)OD wherein D is a group



D

wherein n is an integer from 0 to 3, and

each of R^6 and R^7 is independently hydrogen, $-C_1-C_6$ alkyl, $-C_2-C_6$ alkenyl, $-C_2-C_6$ alkynyl, halogen, -CN, $-CO_2H$, $-CO_2C_1-C_6$ alkyl, $-CONH_2$, $-CONHC_1-C_6$ alkyl, $-CON(C_1-C_6$ alkyl)$_2$, -CHO, $-CH_2OH$, $-CF_3$, $-OC_1-C_6$ alkyl, $-SC_1-C_6$ alkyl, $-SOC_1-C_6$ alkyl, $-SO_2C_1-C_6$ alkyl, $-NH_2$ or -NHCOMe; or

R^1 together with R^2 and the atoms to which they are attached form a 5 to 8 membered nitrogen-containing heterocyclic ring;

R^3 represents hydrogen or halogen;

B represents:

a) a -VR^8 group wherein V is -C(=O)-, -C(=O)O-, -CH_2O-, -CH_2OC(=O)-, -C(=S)-, -CH_2OC(=O)NH-, -C(=S)O-, -CH_2S-, -C(=O)$NHSO_2$- or -SO_2NHC(=O)-; and

R^8 is hydrogen, -C_1-C_{18} alkyl, -C_2-C_{18} alkenyl, -C_2-C_{18} alkynyl, -(C_1-C_6 alkyl)OC_1-C_6 alkyl, -(C_1-C_6 alkyl)SC_1-C_6 alkyl, -(C_1-C_6 alkyl)O(C_1-C_6 alkyl)OC_1-C_6 alkyl, -C_3-C_8 cycloalkyl, -C_4-C_8 cycloalkenyl or pyridyl, (any of which may optionally be substituted with one or more substituents selected from halogen, hydroxyl, nitro, nitrile or carboxyl), -C_1-C_4 perfluoroalkyl, a group -D as defined above or a -(C_1-C_6 alkyl)OD group wherein D is as defined above;

b) a -$CH_2NR^9R^{10}$ group or a -$CONR^9R^{10}$ group wherein each of R^9 and R^{10} is independently hydrogen, -C_1-C_{18} alkyl, -C_2-C_{18} alkenyl, -C_2-C_{18} alkynyl, -C_3-C_8 cycloalkyl, -C_4-C_8 cycloalkenyl, pyridyl (any of which may optionally be substituted with one or more substituents selected from halogen, hydroxyl, nitro, nitrile or carboxyl) or a group -D as defined above or R^9 and R^{10} together with the nitrogen atom to which they are attached form a 5 to 8 membered nitrogen-containing heterocyclic ring;

c) a group Y where Y is a 5- or 6-membered heterocyclic ring containing one or more heteroatoms selected from nitrogen, oxygen and sulphur and the ring may be optionally substituted with one or more substituents selected from -C_1-C_6 alkyl, -OC_1-C_6 alkoxy, halogen, -CF_3 and -CN; or

d) a group -CH_2-Y or C(=O)NHY; where Y is as defined above;

or a pharmaceutically or veterinarily acceptable acid addition salt or hydrate thereof.

Hereafter in this specification the term "compound" includes "salt" or "hydrate" unless the context requires otherwise.

As used herein the term "halogen" or its abbreviation "halo" means fluoro, chloro, bromo or iodo.

As used herein the term "C_1-C_6 alkyl" refers to straight chain or branched chain hydrocarbon groups having from one to six carbon atoms. Illustrative of such alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-

butyl, pentyl, neopentyl and hexyl.

As used herein the term "C_1-C_{18} alkyl" refers to straight chain or branched chain hydrocarbon groups having from one to eighteen carbon atoms. Illustrative of such alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, neopentyl, hexyl, decyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl and octadecyl. From one to six carbon atoms may be preferred.

As used herein the term "C_2-C_6 alkenyl" refers to straight chain or branched chain hydrocarbon groups having from two to six carbon atoms and having in addition one double bond, of either E or Z stereochemistry where applicable. This term would include for example, vinyl, 1-propenyl, 1- and 2-butenyl and 2-methyl-2-propenyl.

As used herein the term "C_2-C_{18} alkenyl" refers to straight chain or branched chain hydrocarbon groups having from two to eighteen carbon atoms and having in addition one or more double bonds, of either E or Z stereochemistry where applicable. This term would include for example, vinyl, 1-propenyl, 1- and 2-butenyl, 2-methyl-2-propenyl, geranyl, and farnesyl. From two to six carbon atoms may be preferred.

As used herein the term "C_2-C_6 alkynyl" refers to straight chain or branched chain hydrocarbon groups having from two to six carbon atoms and having in addition one triple bond. This term would include for example, ethynyl, 1-propynyl, 1- and 2-butynyl, 2-methyl-2-propynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl and 5-hexynyl.

As used herein the term "C_2-C_{18} alkynyl" refers to straight chain or branched chain hydrocarbon groups having from two to six carbon atoms and having in addition one triple bond. This term would include for example, ethynyl, 1-propynyl, 1- and 2-butynyl, 2-methyl-2-propynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, 5-hexynyl, 10-undecynyl, 4-ethyl-1-octyn-3-yl, 7-dodecynyl, 9-dodecynyl, 10-dodecynyl, 3-methyl-1-dodecyn-3-yl, 2-tridecynyl, 11-tridecynyl, 3-tetradecynyl, 7-hexadecynyl and 3-octadecynyl. From two to six carbon atoms may be preferred.

As used herein, the term "C_1-C_4 perfluoroalkyl" refers to straight chain or branched chain groups having from one to four carbon atoms and substituted by

more than one fluorine atom. This term would include for example, trifluoromethyl, 2,2,2-trifluoroethyl, pentafluoroethyl, 3,3,3-trifluoro-n-propyl, sexafluoro-i-propyl, septafluoro-n-propyl, septafluoro-i-propyl, 4,4,4-trifluoro-n-butyl, nonafluoro-n-butyl, nonafluoro-sec-butyl and nonafluoro-i-butyl.

As used herein the term "OC_1-C_6 alkyl" refers to straight chain or branched chain alkoxy groups having from one to six carbon atoms. Illustrative of such alkoxy groups are methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, sec-butoxy, tert-butoxy, pentoxy, neopentoxy and hexoxy.

As used herein the term "SC_1-C_6 alkyl" refers to straight chain or branched chain alkylthio groups having from one to six carbon atoms. Illustrative of such alkyl groups are methylthio, ethylthio, propylthio, isopropylthio, butylthio, isobutylthio, sec-butylthio, tert-butylthio, pentylthio, neopentylthio and hexylthio.

As used herein, the term "C_3-C_8 cycloalkyl" refers to an alicyclic group having from 3 to 8 carbon atoms. Illustrative of such cycloalkyl groups are cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

As used herein, the term "C_4-C_8 cycloalkenyl" refers to an alicyclic group having from 4 to 8 carbon atoms and having in addition one or more double bonds. Illustrative of such cycloalkenyl groups are cyclopentenyl, cyclohexenyl, cycloheptenyl and cyclooctenyl.

As used herein, the term "side chain of a naturally occurring amino acid" includes the side chains of alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glycine, histidine, 5-hydroxylysine, 4-hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, α–aminoadipic acid, α–amino-n-butyric acid, 3,4-dihydroxyphenylalanine, homoserine, α–methylserine, ornithine, pipecolic acid, and thyroxine. The amino acid side chains may be protected; for example the carboxyl groups of aspartic acid, glutamic acid and α–aminoadipic acid may be esterified (for example as a C_1-C_6 alkyl ester), the amino groups of lysine, ornithine, 5-hydroxylysine, 4-hydroxyproline may be converted to amides (for example as a COC_1-C_6 alkyl amide) or carbamates (for example as a $C(=O)OC_1$-C_6 alkyl or $C(=O)OCH_2Ph$ carbamate), the hydroxyl groups of 5-hydroxylysine, 4-hydroxyproline, serine, threonine, tyrosine, 3,4-dihydroxyphenylalanine, homoserine, α–methylserine and thyroxine may be converted to ethers (for example a C_1-C_6 alkyl or a (C_1-C_6 alkyl)phenyl ether)

or esters (for example a C(=O)C1-C6 alkyl ester) and the thiol group of cysteine may be converted to thioethers (for example a C1-C6 alkyl thioether) or thioesters (for example a C(=O)C1-C6 alkyl thioester). The stereochemistry at the carbon atom to which the amino acid side chain is attached may be either D or L.

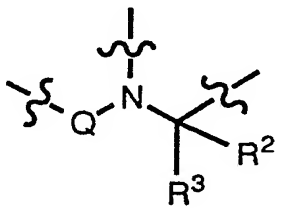
As used herein, the term "nitrogen-containing heterocyclic ring" refers to an aromatic or alicyclic ring comprising one or more nitrogen atoms and optionally one or more other heteroatoms. Illustrative of such rings are pyrrolidine, piperidine, hexamethyleneimine, heptamethylenimine, morpholine and piperazine.

In compounds of this invention, the presence of several asymmetric carbon atoms gives rise to diastereoisomers, each of which consists of two enantiomers, with the appropriate R or S stereochemistry at each chiral center. The invention is understood to include all such diastereoisomers, their optically active enantiomers and mixtures thereof.

The term "pharmaceutically or veterinarily acceptable acid addition salt" refers to a salt prepared by contacting a compound of formula (I) with an acid whose anion is generally considered suitable for human or animal consumption.

Examples of pharmaceutically and/or veterinarily acceptable acid addition salts include the hydrochloride, sulphate, phosphate, acetate, propionate, lactate, maleate, succinate and tartrate salts.

It is considered that the main structural features of compounds of general formula I that are particularly significant in providing their PAF antagonist activity, are the sp^2 nitrogen heterocycle (W group) and the subunit (i)



(i)

The linkage -Z- is considered to function as a spacer element, separating the sp^2 nitrogen heterocycle from the amino acid subunit. The nature or identity of the linkeage -Z- is therefore not thought to be particularly critical and any of the wide range of -Z- groupings specified above may be used with retention of PAF

antagonist activity. Likewise, since the presence of the subunit (i) appears to be crucial for retention of PAF antagonist activity. There may be considerable variation of the substituent groups R^1, and B with out loss of such activity. Any of the the wide range of substituents R^1 and B defined above may be used with retention of PAF antagonist activity.

Of the sp^2 nitrogen heterocycles present in compounds described in our previous patent applications (WO 90/09997, WO 91/17157, WO 92/03422 and WO 92/03423) it is considered that those which are particularly prefered elements of the compounds of this invention are those specified above in relation to general formula I. However, it is expected that PAF antagonist activity may be found in compounds analogous to those of general formula I above, wherein W is a different sp^2 nitrogen heterocycle. The variety of sp^2 nitrogen heterocycles that could provide PAF antagonist activity include those disclosed in our patent application WO 91/17157 and those recently described by Whittaker (Whittaker, M., Curr. Opin. Thera. Patents 2(5), 583-623 (1992)) and Cooper (Cooper, K., *et al.*, J. Med. Chem. 35(17), 3115-3129 (1992)). The exact nature of the interaction of the sp^2 nitrogen heterocycle and the receptor has not been determined, but it would appear that it is important for the heterocycle to possess at least one unsubstituted sp^2 nitrogen atom within the heterocyclic ring.

Preferred compounds include those in which, independently or in any compatible combination;

W represents pyrid-3-yl, 2-methylbenzimidazol-1-yl, 2-methylimidazo[4,5-c]pyridin-1-yl, 2-methylimidazo[4,5-c]pyridin-3-yl, imidazo[4,5-c]pyridin-5-yl and 2-methylimidazo[4,5-c]pyridin-5-yl;

Z represents a) an alkanediyl having from 3 to 11 carbon atoms (for example propylene, 2-hydroxypropylene, 1-methylpropylene, 1,1-dimethylpropylene, butylene, 1-methylbutylene, 1,1-dimethylbutylene, 3-hydroxybutylene, pentylene, 1-methylpentylene, 1,1-dimethylpentylene, 4-hydroxypenylene, 4-methoxypentylene, hexylene, 1,1-dimethylhexylene, heptylene, 1-methylheptylene, 1,1-dimethylheptylene, octylene, 1,1-dimethyloctylene, nonylene, decylene, undecylene) group, an alkenediyl (for example prop-2-enylene, pent-3-enylene hex-5-enylene) group or an alkynediyl (for example prop-2-ynylene, 1-methylprop-2-ynylene, but-3-ynylene, pent-4-ynylene and hex-5-ynylene) group, or;

b) a $-(CH_2)_qU(CH_2)_r-$ group, optionally substituted by nitrile, wherein;

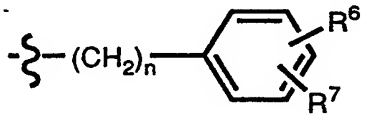
U represents -O-, -S- or a tetrahydrofurandiyl, furandiyl, a thiophenediyl, a piperidinediyl, a piperazinediyl or a benzenediyl group;

q represents an integer of 0, 1, or 2 (provided that, when U is a 1,4-benzenediyl group q is not an integer of 1);

r represents an integer of 0;

R^1 represents a hydrogen atom, a $-C_1-C_6$ alkyl (for example methyl, ethyl) group, a $-C_2-C_6$ alkenyl (for example allyl) group, a $-CO_2C_1-C_6$ alkyl (for example ethoxycarbonyl) group or a $-(C_1-C_6$ alkyl)$CO_2C_1-C_6$ alkyl (for example a ethoxycarbonylmethyl or a t-butyloxycarbonylmethyl) group;

R^2 represents a $-C_1-C_6$ alkyl (for example methyl, isopropyl, n-butyl, isobutyl or 2-methylpropyl) group, a $-C_2-C_6$ alkenyl (for example allyl) group, a $-(C_1-C_6$ alkyl)SC_1-C_6 alkyl (for example methylthioethylene) group, the side chain of a naturally occurring amino acid (for example the side chain of tryptophan), a group -D or a $-(C_1-C_6$ alkyl)OD group;



D

n represents an integer of 0 or 1;

R^6 represents a hydrogen atom or a $-OC_1-C_6$ alkyl (for example methoxy) group;

R^7 represents a hydrogen atom;

R^3 represents a hydrogen atom;

when R^2 represents the side chain of a naturally occurring amino acid, particularly leucine, wherein the stereochemistry of the carbon atom to which R^2 and R^3 are attached is the same as, or the opposite to, that of the naturally occurring amino acid;

B represents a $-VR^8$ group, a $-CONR^9R^{10}$ group or a group Y wherein:

V represents a -C(=O)O- group, a $-CH_2OC(=O)-$ group, a $-CH_2O-$ group, a

-$CH_2OC(=O)$- group or a -$CH_2OC(=O)NH$- group;

R^8 represents a hydrogen atom, -C_1-C_{18} alkyl (for example methyl, ethyl, n-propyl, i-propyl, n-butyl, pentyl, hexyl, octyl, decyl, dodecyl, pentadecyl, hexadecyl, heptadecyl and octadecyl) group, a -C_2-C_{18} alkenyl group (for example allyl), a -(C_1-C_6 alkyl)O(C_1-C_6 alkyl)OC_1-C_6 alkyl (for example a 2-(2-ethoxyethoxy)ethyl) group, a pyridyl (for example a 2-pyridyl) group, a group D or a -(C_1-C_6 alkyl)OD group;

R^9 is a pyridyl (for example 2-pyridyl) group;

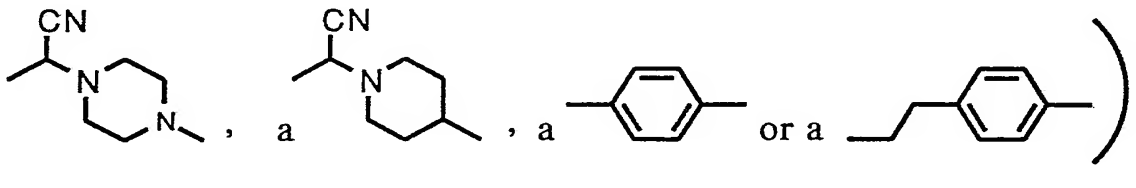
R^{10} is a hydrogen atom;

Y is a pyrazinyl (for example 2-pyrazinyl) group or a oxadiazolyl (for example a 1,2,4-oxadiazol-5-yl) group;

Particularly preferred compounds include those in which, independently or in any compatible combination;

W represents pyrid-3-yl, 2-methylbenzimidazol-1-yl, 2-methylimidazo[4,5-c]pyridin-1-yl, 2-methylimidazo[4,5-c]pyridin-3-yl and 2-methylimidazo[4,5-c]pyridin-5-yl;

Z represents an alkanediyl ((for example propylene, 2-hydroxypropylene, 1-methylpropylene, 1,1-dimethylpropylene, butylene, 1-methylbutylene, 1,1-dimethylbutylene, 3-hydroxybutylene, pentylene, 1-methylpentylene, 1,1-dimethylpentylene, 4-methoxypentylene, hexylene, 1,1-dimethylhexylene, heptylene, 1-methylheptylene, 1,1-dimethylheptylene, octylene, 1,1-dimethyloctylene, nonylene, decylene, undecylene)) group or a -$(CH_2)_qU(CH_2)_r$- (for example a



group;

R^1 represents a hydrogen atom, a -C_1-C_6 alkyl (for example methyl, ethyl) group, a -C_2-C_6 alkenyl (for example allyl) group, or a -CO_2C_1-C_6 alkyl (for example ethoxycarbonyl) group;

Q represents a carbonyl or sulphonyl group;

R^3 represents the side chain of the amino acid leucine;

Exemplary compounds include:

1. (A) N-6-(2-Methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-leucine ethyl ester,
   (B) N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine ethyl ester,
2. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-D-leucine ethyl ester,
3. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-phenylalanine ethyl ester,
4. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine propyl ester,
5. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-norleucine ethyl ester,
6. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-O-benzyl-L-serine methyl ester,
7. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2-methylhexanoyl-L-leucine ethyl ester,
8. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethylhexanoyl-L-leucine ethyl ester,
9. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)-5-hydroxyhexanoyl-L-leucine ethyl ester,
10. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine hexadecyl ester,
11. (A) N-4-(2-Methylimidazo[4,5-c]pyridin-3-yl)butanoyl-L-leucine ethyl ester,
    (B) N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine ethyl ester,
    (C) N-4-(2-Methylimidazo[4,5-c]pyridin-5-yl)butanoyl-L-leucine ethyl ester,
12. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine methyl ester,
13. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-O-methyl-L-tyrosine ethyl ester,
14. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-methionine ethyl ester,
15. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-norleucine n-butyl ester,

16. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethylbutanoyl-L-leucine ethyl ester,
17. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)-3-hydroxybutanoyl-L-leucine ethyl ester,
18. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-valine ethyl ester,
19. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine hexyl ester,
20. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine decyl ester,
21. N-3-(2-Methylbenzimidazol-1-yl)propylsulphonyl-L-leucine ethyl ester,
22. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-alanine ethyl ester,
23. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-isoleucine ethyl ester,
24. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-norleucine ethyl ester,
25. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-methionine ethyl ester,
26. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine i-propyl ester,
27. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine pentyl ester,
28. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine octyl ester,
29. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine dodecyl ester,
30. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine pentadecyl ester,
31. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine hexadecyl ester,
32. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine octadecyl ester,
33. (A) N-4-(2-Methylimidazo[4,5-c]pyridin-3-yl)propylsulphonyl-L-leucinyl ethyl ether,
    (B) N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl ethyl ether,
    (C) N-4-(2-Methylimidazo[4,5-c]pyridin-5-yl)propylsulphonyl-L-leucinyl ethyl ether,
34. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl methyl ether,

35. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl octyl ether,
36. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl hexadecyl ether,
37. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl benzyl ether,
38. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl propionate,
39. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl octadecanoate,
40. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl N'-ethyl carbamate,
41. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl N'-benzyl carbamate,
42. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl N'-2-pyridylcarbamate,
43. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl N-octadecylcarbamate,
44. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-1-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine,
45. (A) N-5-(2-Methylimidazo[4,5-c]pyridin-3-yl)pentanoyl-L-leucine ethyl ester,
    (B) N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucine ethyl ester,
46. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucine i-propyl ester,
47. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-O-methyl-L-tyrosine ethyl ester,
48. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-D,L-allylglycine ethyl ester,
49. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-norleucine allyl ester,
50. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2-methylpentanoyl-L-leucinyl ethyl ether,
51. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethylpentanoyl-L-leucine 2-benzoxyethylethyl ester,
52. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)-3-hydroxypentanoyl-L-leucine 2-(2-ethoxyethoxy)ethyl ester,

53. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-1-(3-methyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine,
54. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-1-(6-ethylpyrazin-2-yl)-3-methylbutylamine,
55. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucinyl N'-ethyl-carbamate,
56. (A) N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-leucine ethyl ester,
(B) N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine ethyl ester,
(C) N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-leucine ethyl ester,
57. (A) N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-isoleucine allyl ester,
(B) N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-isoleucine allyl ester,
(C) N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-isoleucine allyl ester,
58. (A) N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-leucinyl ethyl ether,
(B) N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucinyl ethyl ether,
(C) N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-leucinyl ethyl ether,
59. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucinyl hexadecyl ether,
60. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-phenyl-alaninyl ethyl ether,
61. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucinyl 4-methoxybenzyl ether,
62. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-norleucinyl ethyl ether,
63. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-O-benzyl-L-serinyl ethyl ether,
64. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)-2-methylhexanoyl-L-leucinyl ethyl ether,
65. N-Ethoxycarbonyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucinyl ethyl ether,

66. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)-5-methoxyhexanoyl-L-leucinyl ethyl ether,
67. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-1-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine,
68. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine ethyl ester,
69. N-Allyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine i-propyl ester,
70. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucinyl ethyl ether,
71. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)-2-methylbutanoyl-L-leucinyl ethyl ether,
72. N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucine ethyl ester,
73. N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucinyl ethyl ether,
74. N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)-2-methylpentanoyl-L-leucinyl ethyl ether,
75. N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucinyl hexadecyl ether,
76. N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine ethyl ester,
77. N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine i-propyl ester,
78. N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl ethyl ether,
79. N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl hexadecyl ester,
80. N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-1-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine,
81. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butylsulphonyl-L-leucine ethyl ester,
82. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butylsulphonyl-L-leucinyl ethyl ether,
83. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butylsulphonyl-L-leucinyl heptadecyl ether,
84. N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentylsulphonyl-L-leucine ethyl ester,

85. N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentylsulphonyl-L-leucine i-propyl ester,
86. N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentylsulphonyl-L-leucinyl ethyl ether,
87. (A) N-8-(2-Methylimidazo[4,5-c]pyridin-3-yl)octanoyl-L-leucine ethyl ester,
(B) N-8-(2-Methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucine ethyl ester,
(C) N-8-(2-Methylimidazo[4,5-c]pyridin-5-yl)octanoyl-L-leucine ethyl ester,
88. N-8-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2-methyloctanoyl-L-leucine ethyl ester,
89. N-8-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethyloctanoyl-L-phenyl-alanine ethyl ester,
90. N-Methyl-N-8-(2-methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucine i-propyl ester,
91. N-Methyl-N-8-(2-methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucinyl ethyl ether,
92. N-Methyl-N-8-(2-methylimidazo[4,5-c]pyridin-1-yl)octanoyl-1-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine,
93. N-7-(2-Methylimidazo[4,5-c]pyridin-1-yl)heptanoyl-L-leucine ethyl ester,
94. N-Methyl-N-7-(2-methylimidazo[4,5-c]pyridin-1-yl)heptanoyl-L-leucinyl ethyl ether,
95. N-Methyl-N-7-(2-methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethyl-heptanoyl-L-leucinyl ethyl ether,
96. N-Methyl-N-7-(2-methylimidazo[4,5-c]pyridin-1-yl)heptanoyl-L-leucinyl N'-hexadecylcarbamate,
97. N-11-(2-Methylbenzimidazol-1-yl)undecanoyl-L-leucine ethyl ester,
98. (A) N-11-(2-Methylimidazo[4,5-c]pyridin-3-yl)undecanoyl-L-leucine ethyl ester,
(B) N-11-(2-Methylimidazo[4,5-c]pyridin-1-yl)undecanoyl-L-leucine ethyl ester,
99. N-9-(2-Methylimidazo[4,5-c]pyridin-1-yl)nonanoyl-L-leucine ethyl ester,
100. N-Methyl-N-9-(2-methylimidazo[4,5-c]pyridin-1-yl)nonanoyl-L-leucine i-propyl ester,
101. N-Methyl-N-9-(2-methylimidazo[4,5-c]pyridin-1-yl)nonanoyl-L-leucinyl ethyl ether,
102. N-Methyl-N-9-(2-methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethylnonanoyl-

L-leucinyl ethyl ether,

103. N-Methyl-N-10-(2-methylimidazo[4,5-c]pyridin-1-yl)decanoyl-L-leucinyl ethyl ester,
104. N-Methyl-N-10-(2-methylimidazo[4,5-c]pyridin-1-yl)decanoyl-L-leucine ethyl ester,
105. N-Methyl-N-11-(2-methylimidazo[4,5-c]pyridin-1-yl)undecanoyl-L-leucine ethyl ester,
106. N-Methyl-N-11-(2-methylimidazo[4,5-c]pyridin-1-yl)undecanoyl-L-leucinyl ethyl ether,
107. N-Methyl-N-12-(2-methylimidazo[4,5-c]pyridin-1-yl)dodecanoyl-L-leucinyl ethyl ether,
108. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-D-leucine ethyl ester,
109. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-phenylalanine ethyl ester,
110. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine,
111. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-leucine,
112. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-isoleucine,
113. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine,
114. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-phenylalanine,
115. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine,
116. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-methionine,
117. N-8-(2-Methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucine,
118. N-Methyl-N-8-(2-methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucine,
119. N-Methyl-N-11-(2-methylimidazo[4,5-c]pyridin-1-yl)undecanoyl-L-leucine,
120. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine ethyl ester,
121. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucinyl ethyl ether,
122. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-phenyl-alanine ethyl ester,
123. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine n-butyl ester,
124. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-isoleucine ethyl ester,
125. N-Ethyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine ethyl

ester,

126. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine 2-pyridyl amide,
127. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucinyl N'-ethylcarbamate,
128. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucinyl ethanoate,
129. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-1-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine,
130. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucine ethyl ester,
131. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucinyl ethyl ether,
132. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucine i-propyl ester,
133. N-Ethyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucine ethyl ester,
134. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-norleucinyl ethyl ether,
135. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-1-tetrahydrofuryl-3-methyl-butylamine,
136. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-valine ethyl ester,
137. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-N'-methyl-L-tryptophan ethyl ester,
138. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-O-benzyl-L-serine ethyl ester,
139. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-isoleucinyl ethyl ether,
140. N-4-(3-Pyridylcyanomethyl)piperazinecarbonyl-L-leucine ethyl ester,
141. N-4-(3-Pyridylcyanomethyl)piperazinecarbonyl-L-leucine ethyl ester,
142. N-Methyl-N-4-(3-pyridylcyanomethyl)piperazinecarbonyl-L-leucine ethyl ester,
143. N-Methyl-N-4-(3-pyridylcyanomethyl)piperazinecarbonyl-L-leucinyl ethyl ether,
144. N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-leucine ethyl ester,
145. N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-leucinyl ethyl ether,
146. N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-leucine propyl ester,
147. N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-isoleucine ethyl ester,
148. N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-phenylalanine ethyl ester,
149. N-Ethyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-leucinyl ethyl

ether;

or a salt of such a compound.

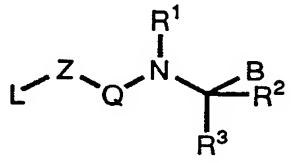
Compounds of general formula I may be prepared by any suitable method known in the art and/or by the following process, which itself forms part of the invention.

According to a second aspect of the invention, there is provided a process for preparing a compound of general formula I as defined above, the process comprising:

(a) treating a nitrogen heterocycle represented by general formula II

W-H II

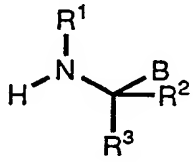
wherein W is as defined in general formula I, with a suitable base (e.g. sodium hydride, potassium hydride or sodium bis(trimethylsilyl)amide), followed by a compound of general formula III



III

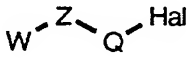
wherein Z, Q, R^1, R^2, R^3 and B are as defined in general formula I, and L is a leaving group such as chloro, bromo, iodo, methanesulphonyloxy, p-toluenesulphonyloxy or trifluoromethanesulphonyloxy;

(b) treating an amine represented by general formula IV



IV

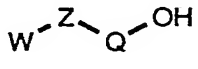
wherein R^1, R^2, R^3, and B are as defined in general formula I, with a suitable base in an aprotic solvent followed by a halo derivative of general formula V



V

wherein W, Z and Q are as defined in general formula I and Hal is a halide such as fluoro, chloro, bromo or iodo;

(c) treating an amine of general formula IV with a derivative of general formula VI



VI

wherein W and Z are as defined in general formula I and Q represents a -C(=O)- group, in the presence of a coupling reagent; and

(d) optionally after step (a), step (b) or step (c) converting, in one or a plurality of steps, a compound of general formula I into another compound of general formula I.

The reaction of step (a) can, for preference, be conducted in an aprotic solvent, for example tetrahydrofuran, to yield compounds of general formula I. The reaction may yield an isomeric mixture, which may be separated by chromatography to yield compounds of general formula I.

The reaction of step (b) can, for preference, be conducted in an aprotic solvent, for example tetrahydrofuran, to yield compounds of general formula I. Suitable bases include sodium hydride, potassium hydride or sodium bis(trimethylsilyl)amide when Q is a bond and triethylamine when Q is a carbonyl, thiocarbonyl or sulphonyl group.

The coupling reagent used in the reaction of step (c) can, for preference, be *N,N'*-dicyclohexylcarbodiimide to yield compounds of general formula I.

By means of step (d), compounds of general formula I wherein B is a $-CO_2R^8$ group can be converted to compounds of general formula I in which B is a $-CO_2H$ group by acid or base catalysed hydrolysis in a protic solvent. Suitable acids for use in the hydrolysis include sulphuric and hydrochloric acids, whilst base hydrolysis can be catalysed with sodium or potassium hydroxide. If B represents a $-CO_2R^8$ group in which R^8 is a benzyl group, the conversion of B from an ester to an acid can also be effected by hydrogenation in a suitable solvent, for example, a lower alcohol such as ethanol using a noble metal catalyst such as palladium or platinum.

Also by means of step (d) compounds of general formula I wherein B is a $-CONR^9R^{10}$ group wherein R^9 and R^{10} are as defined in general formula I, may be prepared by the following methods;

i) by treatment of a compound of general formula I wherein B is a $-CO_2H$ group with an amine of general formula HNR^9R^{10} in the presence of a coupling reagent (e.g. *N,N'*-dicyclohexylcarbodiimide);

ii) by treatment of a compound of general formula I wherein B is a $-CO_2R^8$ group wherein R^8 is a $-C_1$-C_6 alkyl with a dimethylaluminium amide of general formula VII

$$(Me)_2AlNR^9R^{10} \qquad VII$$

wherein R^9 and R^{10} are as defined in general formula I, which is prepared *in situ* from trimethylaluminium and an amine of general formula HNR^9R^{10}.

Also by means of step (d) compounds of general formula I may be prepared by the treatment of a compound of general formula I wherein R^1 is hydrogen with base followed by an electrophile of general formula VIII

$$LR^1 \qquad VIII$$

wherein R^1 is as defined in general formula I but is not a hydrogen atom, a phenyl or a substituted phenyl group, and L is as defined in general formula III. Electrophiles of general formula VIII are available in the art or can be prepared by procedures known to those skilled in the art.

Also by means of step (d) certain compounds of general formula I wherein B is a VR^8 group wherein V is $-CH_2O-$ and R^8 is hydrogen may be prepared by treatment of a compound of general formula I wherein B is a VR^8 group wherein V is $-C(=O)O-$ and R^8 is other than hydrogen with a suitable reducing agent (e.g. lithium aluminium hydride).

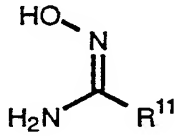
Also by means of step (d) certain compounds of general formula I wherein B is a VR^8 group wherein V is $-CH_2O-$ and R^8 is other than hydrogen may be prepared by treatment of a compound of general formula I wherein B is a VR^8 group wherein V is $-CH_2O-$ and R^8 is hydrogen with a suitable base in an aprotic solvent followed by an electrophile of general formula LR^8 wherein R^8 is $-C_1$-C_{18} alkyl optionally substituted by one or more halogen atoms, $-C_3$-C_{18} alkenyl, $-C_3$-C_{18}

alkynyl, -(C_1-C_6 alkyl)OC_1-C_6 alkyl, -(C_1-C_6 alkyl)SC_1-C_6 alkyl, -(C_1-C_6 alkyl)O(C_1-C_6 alkyl)OC_1-C_6 alkyl, -C_3-C_8 cycloalkyl, -C_4-C_8 cycloalkenyl, a group -D (wherein or n is an integer of 1, 2 or 3) or a -(C_1-C_6 alkyl)OD group and L is a leaving group as defined above.

Also by means of a step (d) certain compounds of general formula I wherein B is a VR^8 group wherein V is a -$CH_2O(C=O)$- group and R^8 is as defined in general formula I but is not hydrogen, may be prepared by treatment of a compound of general formula I wherein B is a VR^8 group wherein V is a -CH_2O- group and R^8 is hydrogen with a compound of general formula $LC(=O)R^8$ wherein L is as defined above and R^8 is as defined in general formula I but is not hydrogen, in an aprotic solvent (e.g. tetrahydrofuran) in the presence of a suitable base (e.g. triethylamine).

Also by means of a step (d) certain compounds of general formula I wherein B is a VR^8 group wherein V is a -$CH_2O(C=O)NH$- group and R^8 is as defined in general formula I but is not hydrogen, may be prepared by treatment of a compound of general formula I wherein B is a VR^8 group wherein V is a -CH_2O- group and R^8 is hydrogen with a compound of general formula $OCNR^8$ wherein R^8 is as defined in general formula I but is not hydrogen.

Also by means of a step (d) certain compounds of general formula I wherein B is a 1,2,4-oxadiazol-5-yl group may be prepared by treatment of a compound of general formula I wherein B is a -CO_2R^8 group wherein R^8 is hydrogen with pentafluorophenol and a coupling agent such as N-(3-dimethylaminopropyl)-N'-ethylcarodiimide in a solvent such as dichloromethane. The resulting pentafluorophenyl ester is treated with an amide oxime of general formula IX

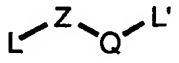


IX

wherein R^{11} represents hydrogen, -C_1-C_6 alkyl, halogen, -CF_3 or -CN, in a suitable aprotic solvent (e.g. chloroform), followed by cyclisation under Dean-Stark conditions in suitable solvent (e.g. xylene, toluene, benzene or ethyl acetate). The cyclisation may be aided by the addition of activated molecular sieves. Amide oximes of general formula IX are known in the art or may be prepared by methods analogous to those known in the art.

Compounds of general formula II are known in the art or may be prepared by methods analogous to those known in the art.

Compounds of general formula III may be prepared by treatment of an amine of general formula IV with an activated carboxylic, thiocarboxylic or sulphonic acid of general formula X

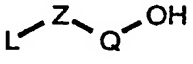


X

wherein Z, and Q are as defined in general formula I, L is as defined above and L' is as defined above for L, in the presence of a suitable base (e.g. triethylamine). Amines of general formula IV and activated carboxylic, thiocarboxylic or sulphonic acids of general formula X are known in the art or may be prepared by methods known in the art.

Compounds of general formula V wherein W, Z and Q are as defined in general formula I, and Hal is chloro may be prepared by the treatment of a compound of general formula VI with thionyl chloride (or oxalyl chloride). The reaction may be aided by the addition of catalytic *N,N*-dimethylformamide.

Compounds of general formula VI may be prepared by the treatment of a compound of general formula II with a suitable base (e.g. sodium hydride, potassium hydride or sodium bis(trimethylsilyl)amide), followed by a compound of general formula XI



XI

wherein Z and Q are as defined in general formula I and L is a leaving group as defined above. Compounds of general formula XI are known in the art or may be prepared by methods known in the art.

The appropriate solvents employed in the above reactions are solvents wherein the reactants are soluble but which do not react with the reactants. The preferred solvents vary from reaction to reaction and are readily ascertained by one of ordinary skill in the art.

Compounds of general formula III, general formula V and general formula VI are valuable intermediates in the preparation of compounds of general formula I, as are other novel compounds specifically or generically disclosed herein. According to a third aspect of the invention, there is therefore provided a

compound of general formula III. According to a fourth aspect of the invention, there is therefore provided a compound of general formula V. According to a fifth aspect of the invention, there is therefore provided a compound of general formula VI.

Compounds of general formula I are potentially useful as PAF antagonists.

This invention therefore also relates to methods of treatment for patients (or animals including mammalian animals raised in the dairy, meat, or fur trades, or as pets) suffering from disorders or diseases which can be attributed to PAF as previously described. More specifically, the invention relates to a method of treatment involving the administration of PAF antagonists of general formula I as the active ingredient. In addition to the treatment of warm blooded animals such as mice, rats, horses, cattle, pigs, sheep, dogs, cats, etc., the compounds of the invention are effective in the treatment of humans.

According to a sixth aspect of the invention there is provided a compound of general formula I for use in human or veterinary medicine particularly in the management of diseases mediated by PAF. When used as PAF antagonists, the compounds of general formula I can be used among other things to reduce inflammation and pain, to correct respiratory, cardiovascular, and intravascular alterations or disorders, and to regulate the activation or coagulation of platelets, to correct hypotension during shock, the pathogenesis of immune complex deposition and smooth muscle contractions.

According to a seventh aspect of the invention there is provided the use of a compound of general formula I in the preparation of an agent for the treatment or prophylaxis of PAF-mediated diseases, and/or the treatment of inflammatory disorders such as rheumatoid arthritis, osteoarthritis and eye inflammation, cardiovascular disorder, thrombocytopenia, asthma, endotoxin shock, adult respiratory distress syndrome, glomerulonephritis, immune regulation, gastric ulceration, transplant rejection, psoriasis, allergic dermatitis, urticaria, multiple sclerosis, cerebral, myocardial and renal ischemia and any other condition in which PAF is implicated.

Compounds of general formula (I) may be administered orally, topically, parenterally, by inhalation spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections,

intravenous, intramuscular, intrasternal injection or infusion techniques.

According to a eighth aspect of the invention there is provided a pharmaceutical or veterinary formulation comprising a compound of general formula I and a pharmaceutically and/or veterinarily acceptable carrier. One or more compounds of general formula I may be present in association with one or more non-toxic pharmaceutically and/or veterinarily acceptable carriers and/or diluents and/or adjuvants and if desired other active ingredients.

The pharmaceutical compositions containing compounds of general formula I may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are

suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occuring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more colouring agents, one or more flavouring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavouring agents may be added to provide a palatable oral preparations. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavouring and colouring agents, may also be present.

Pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavouring and colouring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The compounds of general formula I may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

For topical application to the skin compounds of general formula I may be made up into a cream, ointment, jelly, solution or suspension etc. Cream or ointment formulations that may be used for the drug are conventional formulations well known in the art, for example, as described in standard text books of pharmaceutics such as the British Pharmacopoeia.

For topical applications to the eye, compounds of general formula I may be made up into a solution or suspension in a suitable sterile aqueous or non-aqueous vehicle. Additives, for instance buffers, preservatives including bactericidal and fungicidal agents, such as phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorohexidine, and thickening agents such as hypromellose may also be included.

Compounds of general formula I may be administered parenterally in a sterile medium. The drug depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as a local

anaesthetic, preservative and buffering agents can be dissolved in the vehicle.

Compounds of general formula I may be used for the treatment of the respiratory tract by nasal or buccal administration of, for example, aerosols or sprays which can disperse the pharmacological active ingredient in the form of a powder or in the form of drops of a solution or suspension. Pharmaceutical compositions with powder-dispersing properties usually contain, in addition to the active ingredient, a liquid propellant with a boiling point below room temperature and, if desired, adjuncts, such as liquid or solid non-ionic or anionic surfactants and/or diluents. Pharmaceutical compositions in which the pharmacological active ingredient is in solution contain, in addition to this, a suitable propellant, and furthermore, if necessary, an additional solvent and/or a stabiliser. Instead of the propellant, compressed air can also be used, it being possible for this to be produced as required by means of a suitable compression and expansion device.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per patient per day). For example, inflammation may be effectively treated by the administration of from about 0.01 to 50 mg of the compound per kilogram of body weight per day (about 1.0 mg to about 3.5 g per patient per day). The dosage employed for the topical administration will, of course, depend on the size of the area being treated. For the eyes each dose will be typically in the range from 10 to 100 mg of the drug.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral administration of humans may contain from 0.5 mg to 5 g of active agent compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95 percent of the total composition. Dosage unit forms will generally contain between from about 1 mg to about 500 mg of an active ingredient.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

It has been found that the compounds of general formula I exhibit *in vitro* antagonistic activities with respect to PAF. Compounds of general formula I inhibit PAF-induced functions in both the cellular and tissue levels by changing the PAF binding to its specific receptor site. The ability of compounds of general formula I to inhibit the binding of PAF to its specific receptor binding site on human platelet plasma membranes was measured according to Example 150. The ability of compounds of general formula I to reverse the hypotension caused by an infusion of PAF in rats was measured according Example 151.

The following examples illustrate the invention, but are not intended to limit the scope in any way.

The following abbreviations have been used in the Examples:-

DCM - Dichloromethane
DMAP - 4-Dimethylaminopyridine
DMF - *N,N*-dimethylformamide
TDA-1 - Tris(2-(2-methoxyethoxy)ethyl)amine
THF - Tetrahydrofuran

Unless otherwise stated ^{1}H NMR spectra were recorded on a Bruker AC-250 spectrometer at 250 MHz using $CDCl_3$ as a solvent and internal reference and are reported as delta ppm from TMS.

Example 1

(A) N-6-(2-Methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-leucine ethyl ester and
(B) N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine ethyl ester

(a) N-6-Bromohexanoyl-L-leucine ethyl ester

A stirred suspension of L-leucine ethyl ester hydrochloride (8.38 g, 45 mmol) in dry THF (80 ml) at room temperature was treated with triethylamine (6.3 ml, 45 mmol). The reaction mixture was treated with 6-bromohexanoyl chloride (6.91 ml, 45 mmol). The reaction mixture was stirred for 4 h at room temperature and then diluted with a mixture of saturated aqueous ammonium chloride and ethyl acetate. The organic layer was separated, washed with saturated aqueous ammonium chloride, dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure to give N-6-bromohexanoyl-L-leucine ethyl ester (12.1 g, 80%) as an oil which was used for the next step without further

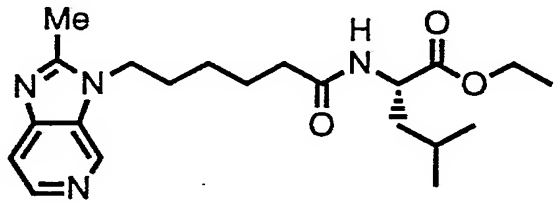
purification.

deltaH 5.94 (1H, d, J 8.1 Hz), 4.70-4.55 (1H, m), 4.16 (2H, q, J 7.2 Hz), 3.39 (2H, t, J 6.4 Hz), 2.22 (2H, t, J 7.2 Hz), 1.94-1.80 (2H, m), 1.74-1.39 (7H, m), 1.26 (3H, t, J 7.0 Hz), 0.93 (6H, d, J 5.8 Hz).

(b) (A) N-6-(2-Methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-leucine ethyl ester and (B) N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine ethyl ester

A stirred solution of 2-methylimidazo[4,5-c]pyridine (3.0 g, 22.5 mmol) in dry THF (60 ml) at room temperature was treated with sodium hydride (900 mg, 22.5 ml). The mixture was stirred for 1 h and the resulting white slurry treated with a solution of N-6-bromohexanoyl-L-leucine ethyl ester (22.5 mmol) in dry THF (30 ml) and stirred overnight. The reaction mixture was diluted with a mixture of saturated aqueous ammonium chloride and ethyl acetate. The organic layer was separated, washed with saturated aqueous ammonium chloride, dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure to give an oil. The crude product mixture was purified by column chromatography (silica: 5% methanol in DCM) and two of the three possible regioisomers were collected, eluting in the order;

(A) N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-leucine ethyl ester

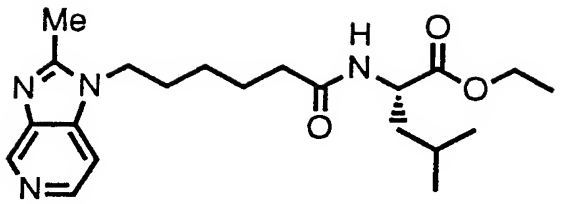


Pale yellow oil (0.7% yield):

i.r. ($CDCl_3$) 1725, 1660 cm^{-1}

deltaH 8.73 (1H, s), 8.39 (1H, d, J 5.3 Hz), 7.58 (1H, d, J 5.5 Hz), 5.94 (1H, br d, J 8.3 Hz), 4.65-4.54 (1H, m), 4.24-4.11 (4H, m), 2.63 (3H, s), 2.21 (2H, t, J 7.2 Hz), 1.90-1.32 (9H, m), 1.27 (3H, t, J 7.3 Hz), 0.93 (3H, d, J 6.0 Hz), 0.92 (3H, d, J 6.3 Hz);

(B) N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine ethyl ester



Pale yellow oil (0.7% yield):

i.r. ($CDCl_3$) 1725, 1660 cm^{-1}

delta$_H$ 8.97 (1H, s), 8.37 (1H, d, J 5.6 Hz), 7.24 (1H, d, 5.5 Hz), 5.92 (1H, br d, J 8.2 Hz), 4.67-4.55 (1H, m), 4.18 (2H, q, J 7.1 Hz), 4.10 (2H, t, J 7.9 Hz), 2.63 (3H, s), 2.22 (2H, t, J 7.2 Hz), 1.90-1.32 (9H, m), 1.28, (3H, t, J 7.2 Hz), 0.94 (3H, d, J 5.9 Hz), 0.93 (3H, d, J 6.2 Hz).

Examples 2-10

The compounds of Examples 2-10 may be prepared by the method of Example 1 employing the appropriate amino acid derivative *in lieu* of L-leucine ethyl ester hydrochloride as starting material and for certain compounds the appropriate substituted 6-bromohexanoyl chloride *in lieu* of 6-bromohexanoyl chloride.

2. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-D-leucine ethyl ester

3. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-phenylalanine ethyl ester

4. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine propyl ester

5. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-norleucine ethyl ester

6. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-O-benzyl-L-serine methyl ester

7. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2-methylhexanoyl-L-leucine ethyl ester

8. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethylhexanoyl-L-leucine ethyl ester

9. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)-5-hydroxyhexanoyl-L-leucine ethyl ester

10. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine hexadecyl ester

Example 11

(A) N-4-(2-Methylimidazo[4,5-c]pyridin-3-yl)butanoyl-L-leucine ethyl ester, (B) N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine ethyl ester and (C) N-4-(2-methylimidazo[4,5-c]pyridin-5-yl)butanoyl-L-leucine ethyl ester

(a) 4-Bromobutanoyl-L-leucine ethyl ester

4-Bromobutanoyl-L-leucine ethyl ester was prepared by the procedure of Example 1 Step (a) employing 4-bromobutanoyl chloride *in lieu* of 6-bromohexanoyl chloride.

Yellow oil (30% yield):

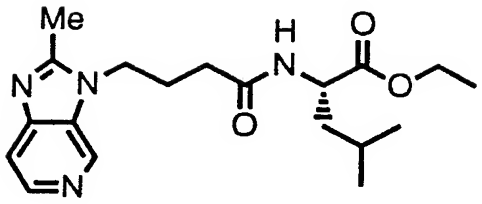
i.r. ($CDCl_3$) 2210, 1730, 1670, 1500, 1155 cm^{-1}

delta$_H$ 6.34 (1H, d, J 8.2 Hz), 4.63-4.50 (1H, m), 4.14 (2H, q, J 7.1 Hz), 3.44 (2H, t, J 6.0 Hz), 2.40 (2H, t, J 7.0 Hz), 2.22-2.07 (2H, m), 1.69-1.44 (3H, m), 1.23 (3H, t, J 7.1 Hz), 0.90 (6H, d, J 5.9 Hz).

(b) (A) N-4-(2-Methylimidazo[4,5-c]pyridin-3-yl)butanoyl-L-leucine ethyl ester, (B) N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine ethyl ester and (C) N-4-(2-methylimidazo[4,5-c]pyridin-5-yl)butanoyl-L-leucine ethyl ester

A suspension of potassium hydroxide (2.1 g, 37 mmol) and TDA-1 (4 drops) in dry acetonitrile (250 ml) under argon was stirred at room temperature for 10 min. 2-Methylimidazo[4,5-c]pyridine (5.0 g, 38 mmol) was added, and the reaction mixture heated at 80°C for 3 h, then cooled to 40°C. A solution of 4-bromobutanoyl-L-leucine ethyl ester (12.0 g, 36 mmol) in dry acetonitrile (250 ml) was added and the reaction mixture stirred at 80°C overnight and cooled to room temperature. Ethanol (100 ml) was added and the resulting slurry filtered through a short pad of celite. Column chromatography (silica: 2-8% methanol in DCM) gave three regioisomeric products eluting in the order;

(A) N-4-(2-methylimidazo[4,5-c]pyridin-3-yl)butanoyl-L-leucine ethyl ester



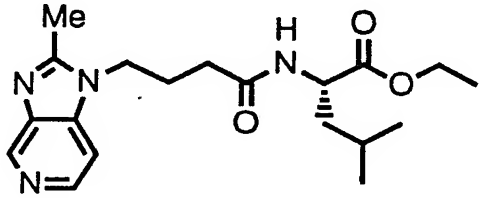
Colourless oil (2% yield):

i.r. ($CDCl_3$) 2210, 1725, 1665, 1500, 1200 cm^{-1}

$delta_H$ 8.69 (1H, s), 8.28 (1H, d, J 5.5 Hz), 7.48 (1H, d, J 5.6 Hz), 7.01 (1H, d, J 8.1 Hz), 4.57-4.48 (1H, m), 4.24-4.18 (2H, m), 4.12 (2H, q, J 7.1 Hz), 2.56 (3H, s), 2.32-2.21 (2H, m), 2.18-2.03 (2H, m), 1.67-1.38 (3H, m), 1.21 (3H, t, J 7.5 Hz), 0.87 (3H, d, J 6.1 Hz), 0.84 (3H, d, J 6.3 Hz);

$delta_C$ 173.10, 171.28, 155.13, 147.57, 141.41, 132.82, 132.14, 113.62, 61.17, 50.80, 43.25, 40.97, 31.86, 25.13, 24.78, 22.60, 21.64, 13.99, 13.58;

(B) N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine ethyl ester



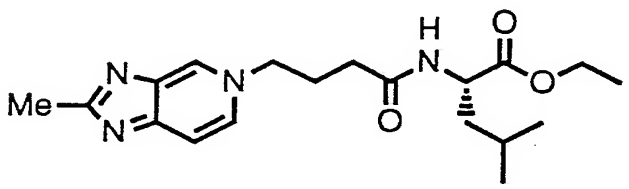
Colourless oil (2% yield):

i.r. ($CDCl_3$) 3445, 2965, 2220, 1730, 1670, 1615, 1510 cm^{-1}

$delta_H$ 8.74 (1H, s), 8.14 (1H, d, J 5.5 Hz), 7.65 (1H, d, J 8.0 Hz), 7.19 (1H, d, J 5.6 Hz), 4.51-4.42 (1H, m), 4.13-3.96 (4H, m), 2.46 (3H, s), 2.23-2.15 (2H, m), 2.07-1.91 (2H, m), 1.61-1.36 (3H, m), 1.34 (3H, t, J 7.1 Hz), 0.80 (3H, d, J 6.1 Hz), 0.75 (3H, d, J 6.2 Hz);

$delta_C$ 172.88, 171.32, 153.32, 140.93, 140.68, 139.95, 139.41, 104.92, 59.67, 49.50, 41.64, 39.42, 30.33, 23.61, 23.42, 21.26, 20.20, 12.65, 12.21;

(C) N-4-(2-methylimidazo[4,5-c]pyridin-5-yl)butanoyl-L-leucine ethyl ester



Yellow oil (5% yield):

i.r. ($CDCl_3$) 2190, 1730, 1665, 1315

delta$_H$ 8.41 (1H, s), 8.12 (1H, d, J 7.7 Hz), 7.66 (1H, d, J 8.2 Hz), 7.49 (1H, d, J 6.7 Hz), 4.50-4.25 (3H, m), 4.11 (2H, q, J 7.1 Hz), 2.56 (3H, s), 2.28-2.02 (4H, m) 1.69-1.44 (3H, m), 1.19 (3H, t, J 7.0 Hz), 0.82 (3H, d, J 6.2 Hz), 0.78 (3H, d, J 6.3 Hz)

delta$_C$ 173.45, 173.14, 171.44, 155.20, 144.55, 130.30, 128.77, 111.32, 60.94, 58.18, 50.99, 39.97, 30.58, 26.93, 24.67, 22.47, 21.21, 17.67, 13.89.

Examples 12-20

The compounds of Examples 12-20 may be prepared by the method of Example 11 employing the appropriate amino acid derivative *in lieu* of L-leucine ethyl ester hydrochloride as starting material and for certain compounds the appropriate substituted 4-bromobutanoyl chloride *in lieu* of 4-bromobutanoyl chloride.

12. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine methyl ester

13. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-O-methyl-L-tyrosine ethyl ester

14. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-methionine ethyl ester

15. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-norleucine n-butyl ester

16. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethylbutanoyl-L-leucine ethyl ester

17. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)-3-hydroxybutanoyl-L-leucine ethyl ester

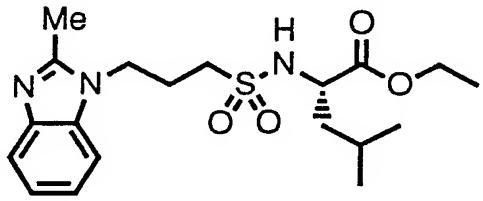
18. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-valine ethyl ester

19. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine hexyl ester

20. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine decyl ester

Example 21

N-3-(2-Methylbenzimidazol-1-yl)propylsulphonyl-L-leucine ethyl ester



N-3-(2-Methylbenzimidazol-1-yl)propylsulphonyl-L-leucine ethyl ester was prepared by the method of Example 1 employing 3-chloropropylsulphonyl chloride *in lieu* of 6-bromohexanoyl chloride and 2-methylbenzimidazole *in lieu* of 2-methylimidazo[4,5-c]pyridine.

Colourles oil (30% yield for last step after chromatography (silica 5% methanol in DCM)):

i.r. ($CDCl_3$) 1730 cm^{-1}

delta$_H$ (250 MHz, $CDCl_3$) 7.73-7.64 (1H, m), 7.85-7.16 (3H, m), 5.42 (1H, d, J 9.6 Hz), 4.26 (2H, t, J 7.2 Hz), 4.21-3.98 (3H, m), 3.07-2.92 (2H, m), 2.60 (3H, s), 2.40-2.22 (2H, m), 1.90-1.70 (1H, m), 1.68-1.46 (2H, m), 1.23 (3H, t, J 7.1 Hz), 0.92 (3H, d, J 6.5 Hz), 0.90 (3H, d, J 6.6 Hz);

delta$_C$ 171.58, 149.87, 140.96, 133.44, 120.96, 120.70, 117.70, 107.57, 60.44, 53.28, 49.08, 40.64, 40.48, 37.10, 23.04, 22.69, 21.35, 19.82, 12.66.

Examples 22-32

The compounds of Examples 22-32 may be prepared by the method of Example 21 employing the appropriate amino acid derivative *in lieu* of L-leucine ethyl ester hydrochloride and 2-methylimidazo[4,5-c]pyridine *in lieu* of 2-methylbenzimidazole as starting materials.

22. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-alanine ethyl ester

23. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-isoleucine ethyl ester

24. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-norleucine ethyl ester

25. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-methionine ethyl ester

26. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine i-propyl ester

27. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine pentyl ester

28. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine octyl ester

29. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine dodecyl ester

30. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine pentadecyl ester

31. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine hexadecyl ester

32. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine octadecyl ester

Example 33

(A) N-4-(2-Methylimidazo[4,5-c]pyridin-3-yl)propylsulphonyl-L-leucinyl ethyl ether, (B) N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl ethyl ether and (C) N-4-(2-methyl-imidazo[4,5-c]pyridin-5-yl)propylsulphonyl-L-leucinyl ethyl ether

(a) L-Leucinyl ethyl ether

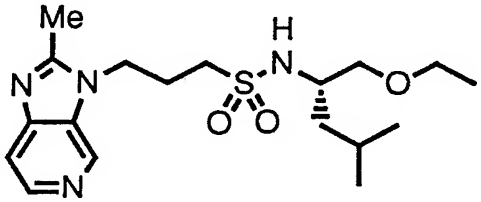
Sodium hydride (60% dispersion in oil: 4.5 g, 0.11 mol) was added to a stirred solution of L-leucinol (12.8 ml, 0.10 mol) in a mixture of dry acetonitrile (24 ml) and dry THF (200 ml) at room temperature under argon. The mixture was heated at reflux for 2 h, cooled to 55°C and ethyl iodide (8.2 ml, 0.10 mol) added carefully. The reaction mixture was heated at reflux overnight and allowed to cool to room temperature. Ice cold brine (100 ml) was added carefully and the mixture extracted with ethyl acetate (3x100 ml). The combined organic extracts were dried over anhydrous sodium sulphate, filtered and evaporated. The residue was distilled under reduced pressure to give L-leucinyl ethyl ether (4.5 g, 30%) as a colourless oil which was used directly in the next step.

delta$_H$ 3.49-3.14 (4H, m), 3.08-2.81 (2H, m), 1.73-1.50 (1H, m), 1.16-0.91 (6H, m), 0.84 (3H, d, J 6.9 Hz), 0.81 (3H, d, J 6.7 Hz).

(b) (A) N-4-(2-Methylimidazo[4,5-c]pyridin-3-yl)propylsulphonyl-L-leucinyl ethyl ether, (B) N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl ethyl ether and (C) N-4-(2-methylimidazo[4,5-c]pyridin-5-yl)propyl-sulphonyl-L-leucinyl ethyl ether

The three regioisomers were prepared by the procedure of Example 11, employing L-leucinyl ethyl ether and 3-chloropropylsulphonyl chloride as starting materials, and were separated by chromatography.

(A) N-4-(2-Methylimidazo[4,5-c]pyridin-3-yl)propylsulphonyl-L-leucinyl ethyl ether



Yellow oil (1% yield for last step after chromatography (silica 4% methanol in DCM)):

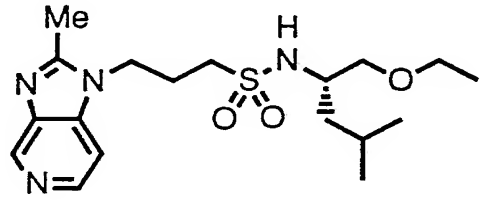
Analysis calculated for $C_{18}H_{30}N_4O_3S.0.7\ H_2O$
Requires C 54.72 H 8.01 N 14.18

Found C 54.72 H 7.91 N 13.87

i.r. ($CDCl_3$) 3395, 2960, 2210, 1610, 1400, 1115 cm^{-1}

delta$_H$ ($CDCl_3$) 8.71 (1H, s), 8.30 (1H, d, J 5.6 Hz), 7.50 (1H, d, J 5.4 Hz), 6.08 (1H, d, J 8.7 Hz), 4.34 (2H, t, J 7.5 Hz), 3.61-3.22 (5H, m), 3.12 (2H, t, J 6.9 Hz), 2.58 (3H, s), 2.36-2.19 (2H, m), 1.77-1.60 (1H, m), 1.40-1.12 (2H, m), 1.03 (3H, t, J 6.9 Hz), 0.84 (3H, d, J 6.3 Hz), 0.81 (3H, d, J 6.4 Hz);

(B) N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl ethyl ether



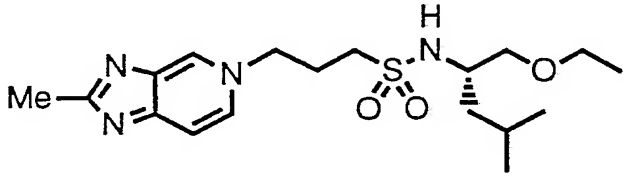
Colourles oil (1% yield):

Analysis calculated for $C_{18}H_{30}N_4O_3S$
Requires C 56.51 H 7.91 N 14.66
Found C 56.25 H 7.86 N 14.50

i.r. ($CDCl_3$) 2215, 1610, 1585, 1390, 1330, 1115 cm^{-1}

delta$_H$ 8.86 (1H, s), 8.23 (1H, d, J 5.5 Hz), 7.25 (1H, d, J 5.6 Hz), 6.26-6.11 (1H, m), 4.27 (2H, t, J 7.3 Hz), 3.63-3.24 (5H, m), 3.10 (2H, t, J 6.7 Hz), 2.57 (3H, s), 2.32-2.16 (2H, m), 1.80-1.62 (1H, m), 1.42-1.16 (2H, m), 1.07 (3H, t, J 7.0 Hz), 0.85 (3H, d, J 6.1 Hz), 0.82 (3H, d, J 6.2 Hz);

(C) N-4-(2-methylimidazo[4,5-c]pyridin-5-yl)propylsulphonyl-L-leucinyl ethyl ether



White crystalline solid (3% yield from ethyl acetate): m.p. 195°C

Analysis calculated for $C_{18}H_{30}N_4O_3S$
Requires C 56.51 H 7.91 N 14.66
Found C 56.29 H 7.81 N 14.58

i.r. ($CDCl_3$) 2195, 1625, 1430, 1320, 1120 cm^{-1}

delta$_H$ ($CDCl_3$) 8.41 (1H, s), 7.60-7.49 (2H, m), 6.74-6.58 (1H, m), 4.55-4.40 (2H, m), 3.66-3.51 (1H, m), 3.48-3.26 (4H, m), 3.09 (2H, t, J 7.1 Hz), 2.69 (3H, s), 2.51-2.36 (2H, m), 1.80-1.63 (1H, m), 1.42-1.14 (2H, m), 1.06 (3H, t, J 7.0 Hz), 0.87 (3H, d, J 6.5 Hz), 0.82 (3H, d, J 6.6 Hz).

Examples 34-44

The compounds of Examples 34-44 may be prepared by the method of Example 33 employing the appropriate amino acid derivative *in lieu* of L-leucinyl ethyl ether as starting material.

34. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl methyl ether

35. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl octyl ether

36. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl hexadecyl ether

37. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl benzyl ether

38. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl propionate

39. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl octadecanoate

40. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl N'-ethyl carbamate

41. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl N'-

benzyl carbamate

42. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl N'-2-pyridylcarbamate

43. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl N-octadecylcarbamate

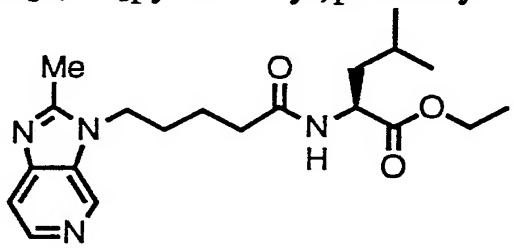
44. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-1-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine

Example 45

(A) N-5-(2-Methylimidazo[4,5-c]pyridin-3-yl)pentanoyl-L-leucine ethyl ester and
(B) N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucine ethyl ester

The compounds of Example 45 were prepared by the procedure of Example 11, utilising 5-bromopentanoyl chloride *in lieu* of 4-bromobutanoyl chloride, and were separated by chromatography.

(A) N-5-(2-methylimidazo[4,5-c]pyridin-3-yl)pentanoyl-L-leucine ethyl ester



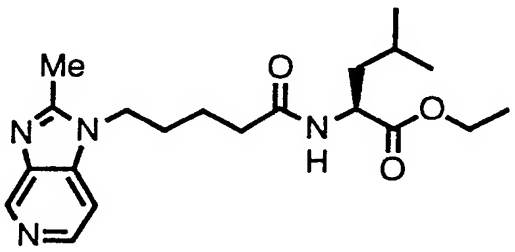
Colourless oil (8% yield for last step after chromatography (silica: 6% methanol in DCM)):

i.r. ($CDCl_3$) 2210, 1730, 1670, 1500, 1400 cm^{-1}

delta$_H$ 8.62 (1H, s), 8.27 (1H, d, J 5.6 Hz), 7.46 (1H, d, J 5.1 Hz), 6.83 (1H, d, J 8.2 Hz), 4.54-4.43 (1H, m), 4.14-4.01 (4H, m), 2.52 (3H, s), 2.18 (2H, t, J 7.0 Hz), 1.86-1.35 (7H, m), 1.15 (3H, t, J 7.1 Hz), 0.81 (3H, d, J 5.9 Hz), 0.79 (3H, d, J 6.1 Hz);

delta$_C$ 172.85, 171.82, 154.60, 147.28, 141.11, 132.50, 131.86, 113.32, 60.67, 50.39, 43.67, 40.56, 34.72, 28.83, 24.47, 22.34, 22.29, 21.27, 13.70, 13.37;

(B) N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucine ethyl ester



Colourless oil (4% yield):

i.r. ($CDCl_3$) 2210, 1730, 1670, 1610, 1510 cm^{-1}

deltaH 8.73 (1H, s), 8.12 (1H, d, J 5.5 Hz), 7.46 (1H, d, J 8.1 Hz), 7.07 (1H, d, J 5.5 Hz), 4.45-4.32 (1H, m), 4.03-3.85 (4H, m), 2.41 (3H, s), 2.18-2.05 (2H, m), 1.72-1.28 (7H, m), 1.04 (3H, t, J 7.1 Hz), 0.71 (3H, d, J 6.3 Hz), 0.67 (3H, d, J 6.4 Hz);

deltaC 172.72, 171.87, 153.04, 140.73, 140.67, 139.64, 139.29, 104.61, 60.59, 50.38, 43.40, 40.47, 34.66, 28.56, 24.42, 22.30, 21.21, 13.65, 13.32.

Examples 46-55

The compounds of Examples 46-55 may be prepared by the method of Example 11 employing the appropriate amino acid derivative *in lieu* of L-leucine ethyl ester hydrochloride as starting material and for certain compounds the appropriate substituted 4-bromopentanoyl chloride *in lieu* of 4-bromopentanoyl chloride.

46. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucine i-propyl ester

47. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-O-methyl-L-tyrosine ethyl ester

48. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-D,L-allylglycine ethyl ester

49. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-norleucine allyl ester

50. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2-methylpentanoyl-L-leucinyl ethyl

ether

51. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethylpentanoyl-L-leucine 2-benzoxyethylethyl ester

52. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)-3-hydroxypentanoyl-L-leucine 2-(2-ethoxyethoxy)ethyl ester

53. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-1-(3-methyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine

54. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-1-(6-ethylpyrazin-2-yl)-3-methylbutylamine

55. N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucinyl N'-ethyl-carbamate

Example 56

(A) N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-leucine ethyl ester, (B) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine ethyl ester and (C) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-leucine ethyl ester

(a) N-Methyl-N-6-bromohexanoyl-L-leucine ethyl ester

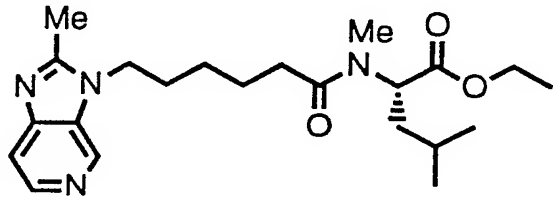
Sodium hydride (60% dispersion in oil; 2.0 g, 50 mmol) was added to a stirred solution of N-6-bromohexanoyl-L-leucine ethyl ester (15.0 g, 45 mmol) in anhydrous THF (150 ml) at 0°C. After the effervesence had ceased methyl iodide (8.4 ml) was added. The reaction mixture was allowed to warm up to room temperature and was stirred overnight. The solvent was removed under reduced pressure and the residue taken up in ethyl acetate, washed with brine, dried over anhydrous sodium sulphate, filtered and concentrated to give crude N-methyl-N-6-bromohexanoyl-L-leucine ethyl ester (14.0 g, 89%) as a pale yellow oil which was used directly in the next step.

deltaH 5.31 (1H, dd, J 10.0, 5.7 Hz), 4.20-4.04 (2H, m), 3.39 (2H, t, J 6.7 Hz), 2.89 (2.5H, s), 2.80 (0.5H, s), 2.41-2.28 (2H, m), 1.95-1.56 (6H, m), 1.54-1.36 (3H, m), 1.23 (3H, t, J 7.1 Hz), 0.92 (3H, d, J 6.2 Hz), 0.89 (3H, d, J 6.1 Hz).

(b) (A) N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hex-anoyl-L-leucine ethyl ester, (B) N-methyl-N-6-(2-methylimidazo-[4,5-c]pyridin-1-yl)hexanoyl-L-leucine ethyl ester and (C) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-leucine ethyl ester

The three regioisomers were prepared by the procedure of Example 11 Step (b), employing N-methyl-N-6-bromohexanoyl-L-leucine ethyl ester *in lieu* of N-3-bromopropanoyl-L-leucine ethyl ester, and were separated by chromatography.

(A) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-leucine ethyl ester



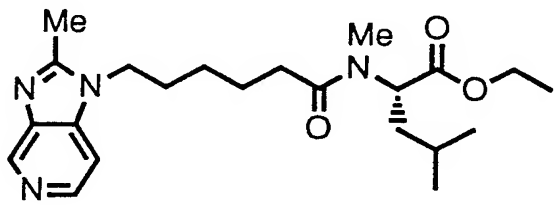
Yellow oil (5% yield for last step after chromatography (silica: 6% methanol in DCM)):

i.r. ($CDCl_3$) 2210, 1725, 1630, 1400, 1195 cm^{-1}

delta$_H$ 8.71 (1H, s), 8.38 (1H, d, J 5.5 Hz), 7.57 (1H, d, J 5.4 Hz), 5.33-5.27 (1H, m), 4.20-4.06 (4H, m), 2.86 (3H, s), 2.62 (3H, s), 2.49-2.26 (2H, m), 1.95-1.80 (2H, m), 1.79-1.62 (4H, m), 1.49-1.32 (3H, m), 1.23 (3H, t, J 7.1 Hz), 0.92 (3H, d, J 6.9 Hz), 0.89 (3H, d, J 6.8 Hz);

delta$_C$ 173.13, 171.98, 154.82, 141.70, 132.25, 113.84, 60.97, 54.18, 44.16, 37.28, 33.05, 31.17, 29.79, 26.51, 25.01, 24.28, 23.16, 21.34, 14.14;

(B) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine ethyl ester



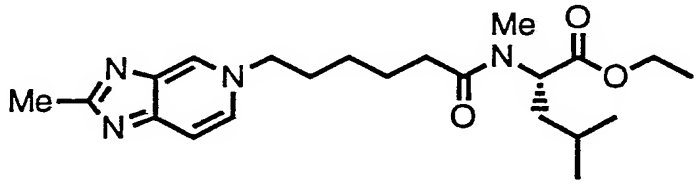
Yellow oil (6% yield):

i.r. ($CDCl_3$) 2210, 1725, 1630, 1610, 1395, 1025 cm^{-1}

deltaH 8.94 (1H, s), 8.35 (1H, d, J 5.6 Hz), 7.23 (1H, d, J 4.9 Hz), 5.32-5.27 (1H, m), 4.18-4.07 (4H, m), 2.86 (3H, s), 2.61 (3H, s), 2.48-2.26 (2H, m), 1.87-1.63 (6H, m), 1.59-1.31 (3H, m), 1.22 (3H, t, J 7.1 Hz), 0.94-0.87 (6H, m);

deltaC 172.91, 171.63, 152.99, 141.14, 141.08, 139.76, 139.45, 104.55, 60.66, 53.95, 43.60, 36.96, 32.74, 30.95, 29.25, 26.16, 24.69, 23.97, 22.88, 21.03, 13.83, 13.58;

(C) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-leucine ethyl ester



Colourless oil (17% yield):

i.r. ($CDCl_3$) 2200, 1730, 1630, 1435, 1320 cm^{-1}

deltaH 8.31 (1H, s), 7.62 (1H, d, J 6.7 Hz), 7.43 (1H, d, J 6.7 Hz), 5.01-4.94 (1H, m), 4.13 (2H, t, J 7.0 Hz), 3.86-3.76 (2H, m), 2.58 (3H, s), 2.43 (3H, s), 2.07-1.99 (2H, m), 1.78-1.61 (2H, m), 1.50-1.30 (4H, m), 1.11-1.10 (3H, m), 0.92 (3H, t, J 7.1 Hz), 0.62 (3H, d, J 6.6 Hz), 0.58 (3H, d, J 6.5 Hz);

deltaC 172.48, 171.20, 153.72, 143.25, 130.37, 129.09, 111.30, 60.28, 59.06, 53.65, 36.59, 32.30, 30.86, 30.62, 25.08, 24.28, 23.25, 22.51, 20.69, 17.06.

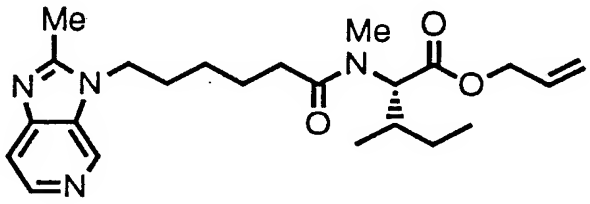
Example 57

(A) N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-isoleucine allyl ester, (B) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-isoleucine allyl ester and (C) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-isoleucine allyl ester

The compounds of Example 57 were prepared by the methods of Example 1 Step

(a) and Example 56 utilising L-isoleucine allyl ester hydrochloride *in lieu* of L-leucine ethyl ester hydrochloride as starting material, and were separated by chromatography.

(A) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-isoleucine allyl ester



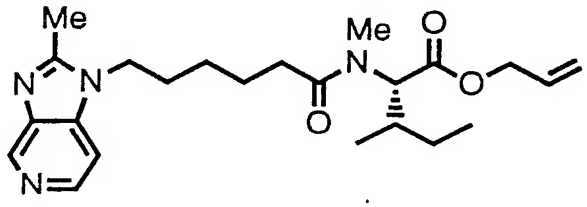
Yellow oil (4% yield for last step after chromatography (silica: 6% methanol in DCM)):

i.r. ($CDCl_3$) 2210, 1730, 1630, 1400, 1180 cm^{-1}

delta$_H$ 8.72 (1H, s), 8.39 (1H, d, J 5.6 Hz), 7.57 (1H, d, J 5.7 Hz), 5.93-5.80 (1H, m), 5.33-5.17 (2H, m), 5.08-5.00 (1H, m), 4.61-4.56 (2H, m), 4.18 (2H, t, J 7.2 Hz), 2.93 (3H, s), 2.63 (3H, s), 2.33 (2H, t, J 7.1 Hz), 2.10-1.81 (3H, m), 1.79-1.63 (2H, m), 1.50-1.04 (4H, m), 0.98-0.76 (6H, m);

delta$_C$ 172.54, 170.38, 154.26, 147.21, 141.26, 135.51, 131.94, 131.34, 117.87, 113.25, 64.66, 59.54, 43.64, 32.98, 32.64, 30.86, 29.30, 26.04, 24.63, 23.82, 15.27, 13.43, 10.17;

(B) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-isoleucine allyl ester



Yellow oil (2% yield):

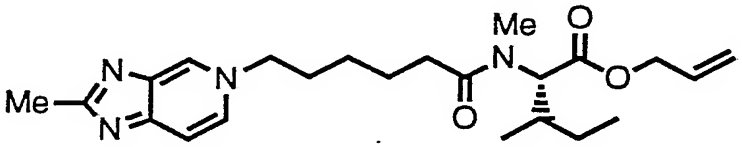
i.r. ($CDCl_3$) 2210, 1730, 1635, 1395, 1160 cm^{-1}

delta$_H$ 8.78 (1H, s), 8.18 (1H, d, J 5.5 Hz), 7.07 (1H, d, J 5.4 Hz), 5.79-5.62

(1H, m), 5.16-5.00 (2H, m), 4.93-4.82 (1H, m), 4.47-4.36 (2H, m), 4.00-3.86 (2H, m), 2.78 (3H, s), 2.44 (3H, s), 2.17 (2H, t, J 7.0 Hz), 1.95-1.75 (1H, m), 1.73-1.45 (4H, m), 1.34-1.10 (4H, m), 0.82-0.59 (6H, m);

deltaC 172.60, 170.41, 152.78, 141.02, 140.95, 139.58, 139.36, 131.34, 117.90, 104.37, 64.69, 59.60, 43.40, 33.02, 32.64, 29.09, 26.01, 24.66, 23.81, 15.30, 15.25, 10.20;

(C) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-isoleucine allyl ester



Yellow oil (8% yield):

i.r. ($CDCl_3$) 2200, 1730, 1630, 1315, 1125 cm^{-1}

deltaH (250 MHz, CDCl3); 8.27 (1H, s), 7.57 (2H, s), 5.89-5.76 (1H, m), 5.28-5.14 (2H, m), 5.04-4.97 (1H, m), 4.59-4.52 (2H, m), 4.27 (2H, t, J 7.1 Hz), 3.44 (3H, s), 2.70 (3H, s), 2.28 (2H, t, J 7.0 Hz), 2.03 (3H, m), 1.74-1.60 (2H, m), 1.41-1.21 (4H, m), 0.97-0.72 (6H, m);

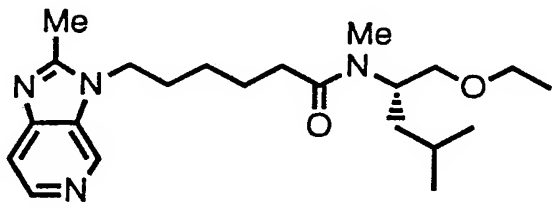
deltaC 174.45, 172.89, 156.01, 145.14, 131.59, 129.32, 128.43, 110.92, 118.35, 111.65, 65.10, 59.97, 59.30, 33.36, 32.84, 31.30, 25.63, 25.00, 23.69, 18.06, 15.55, 10.51.

Example 58

(A) N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-leucinyl ethyl ether, (B) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucinyl ethyl ether and (C) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-leucinyl ethyl ether

The compounds of Example 58 were prepared by the methods of Example 1 Step (a) and Example 56 utilising L-leucinyl ethyl ether *in lieu* of L-leucine ethyl ester hydrochloride as starting material, and were separated by chromatography.

(A) N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-leucinyl ethyl ether



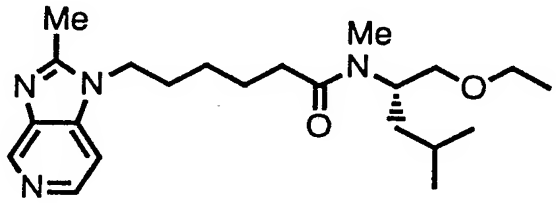
Pale yellow oil (2% yield for last step after chromatography (silica: 5% methanol in DCM)):

i.r. ($CDCl_3$) 2210, 1625, 1400, 1120 cm^{-1}

deltaH 8.60 (1H, s), 8.27 (1H, d, J 5.6 Hz), 7.44 (1H, d, J 5.9 Hz), 4.84-4.71 (0.5H, m), 4.06 (2H, t, J 7.3 Hz), 3.90-3.79 (0.5H, m), 3.42-3.17 (4H, m), 2.68 (1.5H, s), 2.62 (1.5H, s), 2.50 (3H, s), 2.23-2.12 (2H, m), 1.82-1.67 (2H, m), 1.64-1.51 (2H, m), 1.40-1.20 (4H, m), 1.15-0.92 (4H, m), 0.81-0.71 (6H, m);

deltaC 173.02, 172.46, 154.47, 147.43, 141.47, 132.70, 132.11, 113.47, 70.87, 70.55, 66.36, 65.82, 54.34, 49.55, 43.91, 37.90, 37.07, 33.15, 32.29, 29.54, 26.38, 26.29, 26.19, 24.31, 24.23, 24.16, 23.04, 22.97, 22.02, 21.81, 14.86, 14.80, 13.64;

(B) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucinyl ethyl ether



Pale yellow oil (1% yield):

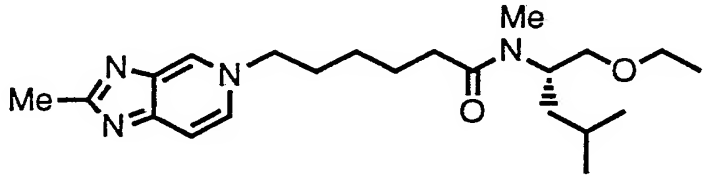
i.r. ($CDCl_3$) 2210, 1620, 1395, 1120 cm^{-1}

deltaH 8.91 (1H, s), 8.31 (1H, d, J 5.6 Hz), 7.20 (1H, d, J 5.7 Hz), 4.91-4.78 (0.5H, m), 4.06 (2H, t, J 7.3 Hz), 3.98-3.87 (0.5H, m), 3.49-3.23 (4H, m), 2.76 (1.5H, s), 2.70 (1.5H, s), 2.57 (3H, s), 2.51-2.39 (0.5H, m), 2.32-2.18 (1.5H, m), 1.84-1.59 (4H, m), 1.48-1.28 (4H, m), 1.23-1.28 (4H, m), 0.90-0.80 (6H,

m);

deltaC .173.23, 172.66, 153.06, 141.52, 141.36, 139.91, 139.67, 104.65, 71.01, 70.71, 66.54, 65.98, 54.52, 49.73, 43.79, 38.06, 37.22, 33.31, 32.43, 29.48, 26.47, 26.41, 24.84, 24.43, 24.28, 23.19, 23.11, 22.16, 21.96, 15.02, 14.95, 13.79;

(C) N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-leucinyl ethyl ether



Brown oil (9% yield):

i.r. ($CDCl_3$) 2195, 1625, 1430, 1120 cm^{-1}

deltaH 8.29 (1H, s), 7.63-7.57 (1H, m), 7.54-7.51 (1H, m), 4.88-4.72 (1H, m), 4.26 (2H, t, J 7.1 Hz), 3.45-3.20 (4H, m), 2.71 (3H, s), 2.65 (3H, s), 2.28-2.15 (2H, m), 1.97-1.82 (2H, m), 1.71-1.55 (2H, m), 1.45-1.17 (5H, m), 1.15-0.95 (3H, m), 0.85-0.75 (6H, m);

deltaC 173.45, 173.36, 172.63, 172.11, 155.27, 144.65, 144.59, 129.40, 129.32, 128.26, 111.10, 70.51, 70.17, 66.01, 65.51, 58.95, 53.99, 49.31, 37.54, 36.75, 32.73, 31.83, 30.96, 29.05, 25.91, 25.35, 25.25, 24.32, 23.93, 23.63, 23.40, 22.73, 22.64, 21.72, 21.50, 17.67.

Examples 59-86

The compounds of Examples 59-86 may be prepared by the method of Example 58 employing the appropriate amino acid derivative *in lieu* of L-leucinyl ethyl ether as starting material and for certain compounds the appropriate substituted haloalkanoyl or haloalkylsulphonylchloride *in lieu* of 6-bromohexanoyl chloride.

59. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucinyl hexadecyl ether

60. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-phenyl-

alaninyl ethyl ether

61. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucinyl 4-methoxybenzyl ether

62. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-norleucinyl ethyl ether

63. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-O-benzyl-L-serinyl ethyl ether

64. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)-2-methylhexanoyl-L-leucinyl ethyl ether

65. N-Ethoxycarbonyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucinyl ethyl ether

66. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)-5-methoxyhexanoyl-L-leucinyl ethyl ether

67. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-1-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine

68. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine ethyl ester

69. N-Allyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine i-propyl ester

70. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucinyl ethyl ether

71. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)-2-methylbutanoyl-L-leucinyl ethyl ether

72. N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucine ethyl ester

73. N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucinyl ethyl ether

74. N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)-2-methylpentanoyl-L-leucinyl ethyl ether

75. N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucinyl hexadecyl ether

76. N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine ethyl ester

77. N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine i-propyl ester

78. N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl ethyl ether

79. N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl hexadecyl ester

80. N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-1-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine

81. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butylsulphonyl-L-leucine ethyl ester

82. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butylsulphonyl-L-leucinyl ethyl ether

83. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butylsulphonyl-L-leucinyl heptadecyl ether

84. N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentylsulphonyl-L-leucine ethyl ester

85. N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentylsulphonyl-L-leucine i-propyl ester

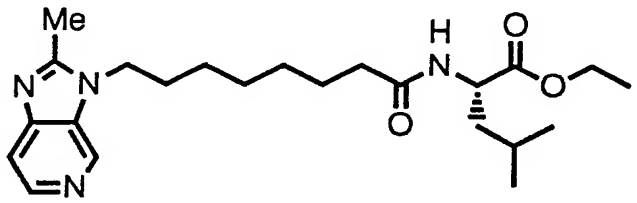
86. N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentylsulphonyl-L-leucinyl ethyl ether

Example 87

(A) N-8-(2-Methylimidazo[4,5-c]pyridin-3-yl)octanoyl-L-leucine ethyl ester, (B) N-8-(2-methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucine ethyl ester and (C) N-8-(2-methylimidazo[4,5-c]pyridin-5-yl)octanoyl-L-leucine ethyl ester

The compounds of Example 87 were prepared by the procedure of Example 11 utilising pentafluorophenyl-8-bromooctanoate *in lieu* of 4-bromobutanoyl chloride, and were separated by chromatography.

(A) N-8-(2-methylimidazo[4,5-c]pyridin-3-yl)octanoyl-L-leucine ethyl ester



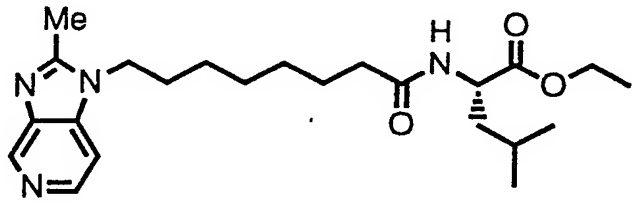
Colourless oil (7% yield for last step after chromatography (silica: 6% methanol in DCM)):

i.r. ($CDCl_3$) 2210, 1730, 1665, 1500, 1400 cm^{-1}

deltaH 8.58 (1H, s), 8.24, (1H, d, J 5.5 Hz), 7.43 (1H, d, 5.3 Hz), 6.68 (1H, d, J 8.2 Hz), 4.51-4.40 (1H, m), 4.06-3.95 (4H, m), 2.49 (3H, s), 2.06 (2H, t, J 7.4 Hz), 1.76-1.60 (2H, m), 1.58-1.34 (5H, m), 1.27-1.10 (6H, m), 1.11 (3H, t, J 7.4 Hz), 0.78 (3H, d, J 5.7 Hz), 0.76 (3H, d, J 5.7 Hz);

deltaC 172.98, 172.54, 154.50, 147.37, 141.33, 132.66, 132.03, 113.44, 60.75, 50.32, 43.94, 41.00, 35.73, 29.44, 28.51, 26.25, 25.00, 24.53, 22.46, 21.50, 13.79, 13.54;

(B) N-8-(2-methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucine ethyl ester



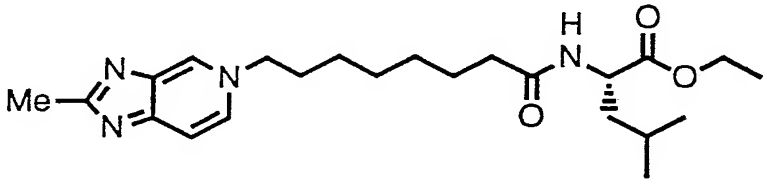
Colourless oil (8% yield):

i.r. ($CDCl_3$) 2210, 1730, 1670, 1500 cm^{-1}

deltaH 8.78 (1H, s), 8.19 (1H, d, J 5.5 Hz), 7.08 (1H, d, J 5.5 Hz), 6.90 (1H, d, J 8.2 Hz), 4.51-4.38 (1H, m), 4.05-3.83 (4H, m), 2.45 (3H, s), 2.06 (2H, t, J 7.3 Hz), 1.67-1.30 (7H, m), 1.23-1.07 (6H, m), 1.08 (3H, t, J 7.1 Hz), 0.75 (3H, d, J 5.9 Hz), 0.73 (3H, d, J 6.0 Hz);

deltaC 172.89, 172.60, 152.96, 140.96, 139.70, 139.40, 104.54, 60.66, 50.30, 43.66, 40.88, 35.64, 29.18, 28.44, 26.19, 24.97, 24.47, 22.41, 21.43, 13.74, 13.48;

(C) N-8-(2-methylimidazo[4,5-c]pyridin-5-yl)octanoyl-L-leucine ethyl ester



Colourless oil (7% yield):

i.r. ($CDCl_3$) 2200, 1730, 1665, 1500, 1435 cm^{-1}

deltaH 8.30 (1H, s), 7.50-7.48 (2H, m), 7.08 (1H, d, J 8.1 Hz), 4.52-4.41 (1H, m), 4.15 (2H, t, J 7.3 Hz), 4.04 (2H, q, J 7.1 Hz), 2.61 (3H, s), 2.06 (2H, t, J 7.3 Hz), 1.87-1.73 (2H, m), 1.65-1.40 (5H, m), 1.23-1.10 (9H, m), 0.80 (3H, d, J 6.4 Hz), 0.78 (3H, d, J 6.5 Hz);

deltaC 174.39, 173.23, 172.72, 150.04, 145.21, 129.09, 128.37, 111.50, 60.81, 59.44, 50.51, 40.88, 35.62, 31.78, 31.28, 28.40, 28.23, 25.63, 24.95, 24.62, 22.53, 21.50, 18.06, 13.86.

Examples 88-96

The compounds of Examples 88-96 may be prepared by the method of Example 11 or Example 56 employing the appropriate amino acid derivative and the appropriate substituted haloalkanoyl chloride as starting materials.

88. N-8-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2-methyloctanoyl-L-leucine ethyl ester

89. N-8-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethyloctanoyl-L-phenyl-alanine ethyl ester

90. N-Methyl-N-8-(2-methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucine i-propyl ester

91. N-Methyl-N-8-(2-methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucinyl ethyl ether

92. N-Methyl-N-8-(2-methylimidazo[4,5-c]pyridin-1-yl)octanoyl-1-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine

93. N-7-(2-Methylimidazo[4,5-c]pyridin-1-yl)heptanoyl-L-leucine ethyl ester

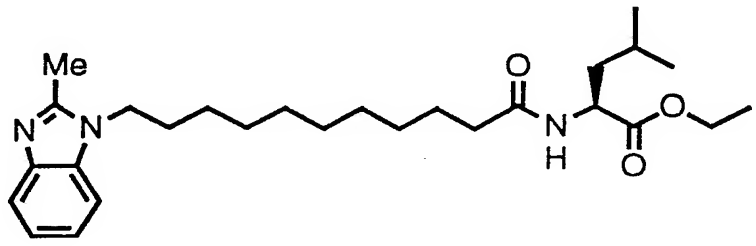
94. N-Methyl-N-7-(2-methylimidazo[4,5-c]pyridin-1-yl)heptanoyl-L-leucinyl ethyl ether

95. N-Methyl-N-7-(2-methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethylheptanoyl-L-leucinyl ethyl ether

96. N-Methyl-N-7-(2-methylimidazo[4,5-c]pyridin-1-yl)heptanoyl-L-leucinyl N'-hexadecylcarbamate

Example 97

N-11-(2-Methylbenzimidazol-1-yl)undecanoyl-L-leucine ethyl ester



N-11-(2-Methylbenzimidazol-1-yl)undecanoyl-L-leucine ethyl ester was prepared by the procedure of Example 1 utilising pentafluorophenyl-11-bromoundecanoate *in lieu* of 6-bromohexanoyl chloride and 2-methylbenzimidazole *in lieu* of 2-methylimidazo[4,5-c]pyridine.

Colourless oil (30% yield for last step after chromatography (silica: 4% methanol in DCM)):

i.r. ($CDCl_3$) 2200, 1730, 1665, 1500, 1400 cm^{-1}

deltaH 7.63-7.58 (1H, m), 7.23-7.11 (3H, m), 6.42 (1H, d, J 7.7 Hz), 4.61-4.52 (1H, m), 4.11 (2H, q, J 6.7 Hz), 3.99 (2H, t, J 7.3 Hz), 2.52 (3H, s), 2.14 (2H, t, J 7.5 Hz), 1.79-1.40 (7H, m), 1.34-1.05 (15H, m), 0.88 (3H, d, J 5.3 Hz), 0.86 (3H, d, J 5.3 Hz);

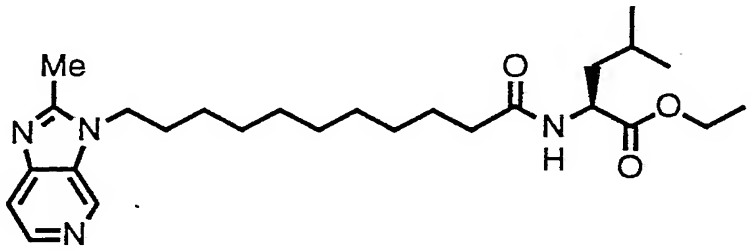
deltaC 173.04, 172.76, 151.12, 142.33, 134.85, 121.61, 121.39, 118.64, 108.94, 60.88, 53.22, 50.41, 43.57, 41.29, 36.12, 29.44, 29.01, 28.93, 26.60, 25.31, 24.63, 24.38, 22.56, 21.69, 13.89, 13.61.

Example 98

(A) N-11-(2-Methylimidazo[4,5-c]pyridin-3-yl)undecanoyl-L-leucine ethyl ester and (B) N-11-(2-methylimidazo[4,5-c]pyridin-1-yl)undecanoyl-L-leucine ethyl ester

The compounds of Example 98 were prepared by the procedure of Example 11 utilising pentafluorophenyl-11-bromoundecanoate *in lieu* of 4-bromobutanoyl chloride, and were separated by chromatography.

(A) N-10-(2-Methylimidazo[4,5-c]pyridin-3-yl)undecanoyl-L-leucine ethyl ester



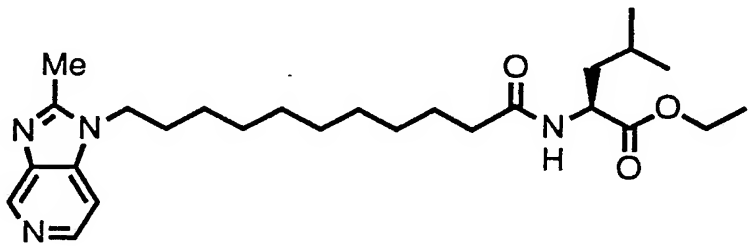
Colourless oil (5% yield for last step after chromatography (silica: 5% methanol in DCM)):

i.r. ($CDCl_3$) 1730, 1665 cm^{-1}

deltaH 8.66 (1H, br s), 8.32 (1H, br s), 7.50 (1H, d, J 5.3 Hz), 6.47 (1H, d, J 8.3 Hz), 4.60-4.50 (1H, m), 4.16-4.02 (4H, m), 2.57 (3H, s), 2.14 (2H, t, J 7.3 Hz), 1.82-1.69 (2H, m), 1.68-1.40 (5H, m), 1.36-1.10 (15H, m), 0.85 (3H, d, J 6.1 Hz), 0.84 (3H, d, J 6.2 Hz);

deltaC 173.12, 172.81, 154.62, 147.56, 141.50, 132.84, 132.15, 113.62, 60.93, 50.42, 44.13, 41.35, 36.21, 29.60, 28.95, 28.83, 26.51, 25.34, 24.68, 22.61, 21.71, 13.93, 13.73;

(B) N-11-(2-methylimidazo[4,5-c]pyridin-1-yl)undecanoyl-L-leucine ethyl ester



Colourless oil (5% yield):

i.r. ($CDCl_3$) 1730, 1670 cm^{-1}

deltaH ($CDCL_3$)cm^{-1} 8.93 (1H, br s), 8.34 (1H, br s), 7.20 (1H, d, J 5.3 Hz), 6.20 (1H, d, J 8.3 Hz), 4.64-4.53 (1H, m), 4.13 (2H, q, J 7.1 Hz), 4.06 (2H, t, J 7.4 Hz), 2.59 (3H, s), 2.16 (2H, t, J 7.3 Hz), 1.80-1.64 (2H, m), 1.66-1.41 (5H, m), 1.34-1.14 (15H, m), 0.90 (3H, d, J 6.0 Hz), 0.89 (3H, d, J 6.2 Hz);

deltaC 173.19, 172.78, 153.16, 141.51, 141.33, 140.01, 104.73, 61.09, 50.51, 44.03, 41.58, 36.34, 29.55, 29.12, 29.03, 28.98, 26.69, 25.41, 24.78, 22.68, 21.87, 14.01, 13.82.

Examples 99-109

The compounds of Examples 99-109 may be prepared by the method of Example 11 or Example 56 employing the appropriate amino acid derivative and the appropriate substituted haloalkanoyl chloride or pentafluorophenyl haloalkanoate as starting materials.

99. N-9-(2-Methylimidazo[4,5-c]pyridin-1-yl)nonanoyl-L-leucine ethyl ester

100. N-Methyl-N-9-(2-methylimidazo[4,5-c]pyridin-1-yl)nonanoyl-L-leucine i-propyl ester

101. N-Methyl-N-9-(2-methylimidazo[4,5-c]pyridin-1-yl)nonanoyl-L-leucinyl

ethyl ether

102. N-Methyl-N-9-(2-methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethylnonanoyl-L-leucinyl ethyl ether

103. N-Methyl-N-10-(2-methylimidazo[4,5-c]pyridin-1-yl)decanoyl-L-leucinyl ethyl ester

104. N-Methyl-N-10-(2-methylimidazo[4,5-c]pyridin-1-yl)decanoyl-L-leucine ethyl ester

105. N-Methyl-N-11-(2-methylimidazo[4,5-c]pyridin-1-yl)undecanoyl-L-leucine ethyl ester

106. N-Methyl-N-11-(2-methylimidazo[4,5-c]pyridin-1-yl)undecanoyl-L-leucinyl ethyl ether

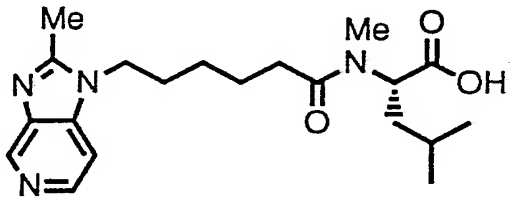
107. N-Methyl-N-12-(2-methylimidazo[4,5-c]pyridin-1-yl)dodecanoyl-L-leucinyl ethyl ether

108. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-D-leucine ethyl ester

109. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-phenylalanine ethyl ester

Example 110

N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine



2M Potassium hydroxide (2.5 ml) was added to a solution of N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine ethyl ester (200 mg, 0.50 mmol) in ethanol (70 ml). The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and water was added to the residue. The pH of the resulting solution was adjusted to pH 6 by the

addition of 2M HCl, the mixture was saturated with sodium chloride and extracted with DCM. The combined organic extracts were dried over anhydrous sodium sulphate, filtered and evaporated to give N-methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine (70 mg, 38%) as a white foam.

i.r. ($CDCl_3$) 2590, 2215, 1720, 1640, 1400, 1315 cm^{-1}

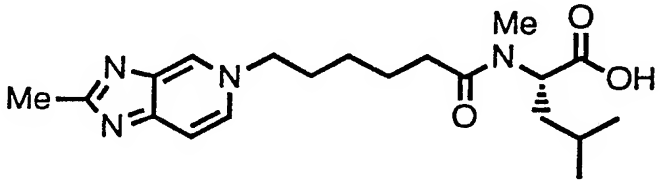
$delta_H$ 9.51 (1H, s), 8.40 (1H, d, J 6.4 Hz), 7.89 (1H, d, J 6.4 Hz), 5.18 (1H, dd, J 10.4 Hz, 5.4 Hz), 4.48-4.31 (2H, m), 2.83 (3H, s), 2.75 (3H, s), 2.65-2.18 (2H, m), 1.93-1.30 (9H, m), 0.86 (3H, d, J 6.8 Hz), 0.82 (3H, d, J 6.5 Hz);

$delta_C$ (CD_3OD) 178.57, 177.27, 167.89, 155.81, 137.08, 130.86, 119.00, 58.23, 40.82, 40.83, 36.40, 34.64, 32.79, 29.67, 28.61, 27.94, 26.09, 24.11.

Examples 111 and 112

The compounds of Examples 111 and 112 were prepared by the procedure of Example 110 starting from the compounds of Examples 56(C) and 57(A) respectively.

111. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-leucine



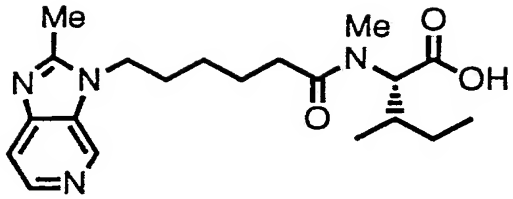
Yellow solid (29% yield): m.p. 80°C

i.r. ($CDCl_3$) 2210, 1720, 1625, 1400, 1125 cm^{-1}

$delta_H$ 9.20 (1H, s), 8.50 (1H, d, J 6.5 Hz), 8.00 (1H, d, J 6.7 Hz), 5.05 (1H, dd, J 10.2, 5.2 Hz), 4.80-4.57 (2H, m), 2.91 (3H, s), 2.75 (3H, s), 2.57-2.21 (2H, m), 2.10-1.91 (2H, m), 1.90-1.25 (7H, m), 0.90 (3H, d, J 6.5 Hz), 0.86 (3H, d, J 6.5 Hz);

$delta_C$ (CD_3OD) 178.29, 166.23, 150.20, 142.98, 140.24, 138.00, 114.99, 64.50, 58.79, 41.18, 36.50, 34.95, 34.77, 29.18, 28.74, 27.79, 26.34, 24.33.

112. N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-isoleucine



White foam (54% yield after chromatography (silica: 6% methanol and 0.5% acetic acid in DCM):

i.r. ($CDCl_3$) 2230, 1715, 1630, 1400, 1135 cm^{-1}

deltaH 8.85 (1H, s), 8.37 (1H, d, J 5.3 Hz), 7.67 (1H, d, J 5.5 Hz), 4.97 (1H, t, J 10.3 Hz), 4.20 (2H, t, J 7.2 Hz), 2.97 (3H, s), 2.66 (3H, s), 2.67-2.52 (1H, m), 2.34 (2H, t, J 6.9 Hz), 2.14-1.54 (6H, m), 1.52-1.10 (3H, m), 1.05-0.76 (6H, m);

deltaC 173.25, 173.15, 156.57, 148.22, 139.30, 132.66, 130.50, 114.13, 44.38, 33.36, 32.71, 29.57, 26.35, 24.41, 24.12, 15.89, 15.08.

Examples 113-119

The compounds of Examples 113-119 may be prepared by the hydrolysis of the appropriate amino acid ester derivative according to the method of Example 110.

113. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine

114. N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-phenylalanine

115. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine

116. N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-methionine

117. N-8-(2-Methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucine

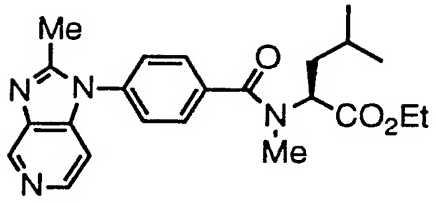
118. N-Methyl-N-8-(2-methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucine

119. N-Methyl-N-11-(2-methylimidazo[4,5-c]pyridin-1-yl)undecanoyl-L-leucine

Example 120

N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine ethyl

ester



(a) 4-(2-Methylimidazo[4,5-c]-pyridin-1-yl)benzoic acid

Sodium hydroxide (0.39 g, 9.7 mmol) was added to water (5 ml) and the resultant solution added to a stirred solution of 4-(2-methylimidazo[4,5-c]-pyridin-1-yl)phenylnitrile [Cooper K. *et al.*, J. Med. Chem. 35(17), 3115-3129 (1992)](224 mg, 0.96 mmol). The mixture was heated under reflux for 1.5 h, cooled, concentrated under reduced pressure and concentrated to dryness to give a brown solid. The residue was taken up in water, neutralised to pH 7 and the resultant mixture passed down an acidic ion exchange column (eluting with 1-30% aqueous ammonia) to give 4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoic acid (130 mg, 53%) as an amorphous solid.

deltaH (400 MHz) 8.93 (1H, d, J 0.9 Hz), 8.31 (1H, d, J 5.5 Hz), 8.10 (2H, d, J 8.5 Hz), 7.51 (2H, d, J 8.5 Hz), 7.22 (1H, dd, J 5.0, 1.0 Hz), 2.51 (3H, s).

(b) N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine ethyl ester

Oxalyl chloride (200 µl, 2.3 mmol) was added dropwise to a stirred suspension of 4-(2-methylimidazo[4,5-c]-pyridin-1-yl)benzoic acid (130 mg, 0.5 mmol) in dry DCM (10 ml) at 0°C under argon. DMF (3 drops) was added and the mixture allowed to warm up to ambient temperature slowly. After 1.5 h the reaction mixture was evaporated to dryness under reduced pressure. Dry DCM (10 ml) was added to the residue and L-leucine ethyl ester hydrochloride (142 mg, 0.72 mmol) and triethylamine (280 µl) added to the resulting solution. The mixture was stirred overnight at ambient temperature and concentrated under reduced pressure. The residue was taken up in ethyl acetate and washed with saturated aqueous sodium hydrogen carbonate and brine. The combined organics were dried over anhydrous magnesium sulphate, filtered and concentrated. Column chromatography (silica: 3% methanol in DCM) gave N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine ethyl ester (47 mg, 23%) as a pale yellow oil.

deltaH (400 MHz) 9.06 (1H, s), 8.50 (1H, d, J 5.6 Hz), 8.04 (2H, d, J 8.6 Hz), 7.45 (2H, d, J 8.6 Hz), 7.07 (1H, dd, J 5.6, 1.0 Hz), 6.66 (1H, d, J 8.3 Hz), 4.87 (1H, m), 4.25 (2H, q, J 7.1 Hz), 2.55 (3H, s), 1.85-1.60 (3H, m), 1.32 (3H, t, J 7.1 Hz), 1.03 (3H, d, J 6.1 Hz), 1.00 (3H, d, J 6.3 Hz).

(c) N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine ethyl ester

A solution of N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine ethyl ester (71 mg, 0.18 mmol) in dry THF (3.5 ml) was added *via* cannula to a stirred suspension of sodium hydride (60% dispersion in oil: 10 mg, 0.25 mmol) in dry THF (1 ml) at room temperature under argon. The mixture was stirred for 0.5 h and dimethyl sulphate (24 µl, 0.25 mmol) added. The mixture was stirred overnight, aqueous ammonium chloride added, the mixture extracted with ethyl acetate and the organics washed with water and brine. The combined organics were dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure. Column chromatography (silica: 3% methanol in DCM) gave N-methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine ethyl ester (23 mg, 31%) as a pale yellow oil.

deltaH (400 MHz) 9.06 (1H, s), 8.40 (1H, d, J 5.6 Hz), 8.04 (2H, d, J 7.8 Hz), 7.43 (2H, d, J 8.0 Hz), 7.11 (1H, m), 5.38 (0.6H, m), 4.37 (0.4H, m), 4.24 (2H, br m), 3.03 (1.2H, s), 2.99 (1.8H, s), 2.56 (3H, s), 1.90-1.47 (3H, m), 1.32 (3H, t, J 7.1 Hz), 1.04 (3.6H, d, J 6.3 Hz), 0.89 (1.2H, d, J 5.0 Hz), 0.71 (1.2H, d, J 5.5 Hz).

Examples 121-129

The compounds of Examples 121-129 may be prepared by the method of Example 120 employing the appropriate amino acid derivative *in lieu* of L-leucine ethyl ester hydrochloride as starting material.

121. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucinyl ethyl ether

122. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-phenylalanine ethyl ester

123. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine n-

butyl ester

124. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-isoleucine ethyl ester

125. N-Ethyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine ethyl ester

126. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine 2-pyridyl amide

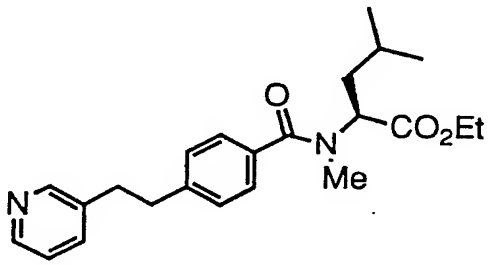
127. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucinyl N'-ethylcarbamate

128. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucinyl ethanoate

129. N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-1-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine

Example 130

N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucine ethyl ester



(a) Methyl 4-(2-(3-pyridyl)ethenyl)benzoate

Solid potassium t-butoxide (3.42 g, 30.5 mmol) was added in one portion to a stirred suspension of (3-pyridyl)methyltriphenylphosphonium chloride hydrochloride (5.20 g, 12.2 mmol) in DMF (90 ml) at 50°C under argon. After 5 min a solution of methyl 4-formylbenzoate (2.0 g, 12.2 mmol) in DMF (10 ml) was added *via* cannula. The reaction mixture was stirred overnight at 50°C, and concentrated under reduced pressure. The residue was dissolved in ethyl acetate (250 ml) and washed with water (100 ml). The organics were extracted with 2M

HCl (2x100 ml) and the combined acidic aqueous layers washed with ethyl acetate (100 ml) then basified to pH 10 by the addition of aqeous sodium hydroxide followed by potassium carbonate. The mixture was extracted with ethyl acetate (2x200 ml) and the combined organics dried over anhydrous magnesium sulphate, filtered and evaporated to give a 30:70 mixture of *trans*- and *cis*-methyl 4-(2-(3-pyridyl)ethenyl)benzoate (2.2 g, 75%) as a brown gum which crystallised on standing.

deltaH (400 MHz) 8.74 (0.3H, d, J 2.0 Hz), 8.51 (0.3H, dd, 4.8, 1.6 Hz), 8.45 (0.7H, d, J 2.2 Hz), 8.42 (0.7H, dd, 4.8, 1.6 Hz), 8.03 (0.6H, d, J 8.4 Hz), 7.90 (1.4H, d, J 8.4 Hz), 7.83 (0.3H, dt, J 8.0, 1.8 Hz), 7.57 (0.6H, d, J 8.5 Hz), 7.46 (0.7H, dt, J 7.9, 1.8 Hz), 7.29 (0.3H, dd, J 7.9, 4.8 Hz), 7.26 (1.4H, d, J 8.2 Hz), 7.17 (0.6H, s), 7.11 (0.7H, dd, J 7.9, 4.7 Hz), 6.75 (0.7H, d, J 12.2 Hz), 6.64 (0.7H, d, J 12.2 Hz), 3.92 (0.9H, s), 3.89 (2.1H, s).

(b) Methyl 4-(2-(3-pyridyl)ethyl)benzoate

Hydrogen gas was slowly bubbled through a vigorously stirred mixture of methyl 4-(2-(3-pyridyl)ethenyl)benzoate (952 mg, 3.98 mmol) and 10% palladium on carbon (95 mg) at room temperature for 4 h. The reaction mixture was filtered through Kieselguhr which was then washed with excess ethyl acetate. The combined organics were evaporated under reduced pressure to give methyl 4-(2-(3-pyridyl)ethyl)benzoate (900 mg, 94%) as an oil which crystallised on standing.

deltaH (400 MHz) 8.43 (1H, dd, J 4.8, 1.6 Hz), 8.40 (1H, d, J 2.0 Hz), 7.93 (2H, d, J 8.4 Hz), 7.39 (1H, dt, J 7.7, 2.0 Hz), 7.18 (2H, d, J 8.4 Hz), 7.15 (1H, dd, J 7.8, 4.8 Hz), 3.89 (3H, s), 2.95 (4H, m).

(c) N-4-(2-(3-Pyridyl)ethyl)benzoyl-L-leucine ethyl ester

Methyl 4-(2-(3-pyridyl)ethyl)benzoate was dissolved in methanol (10 ml) and a solution of potassium hydroxide (2.1 g, 37.3 mmol) in water (2 ml) added. The mixture was stirred overnight and the pH lowered to ca. 7 by the cautious addition of concentrated hydrochloric acid. Methanol (20 ml) was added and the suspension filtered, the filtrate concentrated to give an oil to which 2M HCl was added to bring the pH to 8. A solid precipitate formed, the solvent was removed under reduced pressure and the residue azeotroped with toluene (1x100 ml, 1x50 ml) to give crude 4-(2-(3-pyridyl)ethyl)benzoic acid. Thionyl chloride (10 ml)

and dry DMF (100 µl) were added to the crude 4-(2-(3-pyridyl)ethyl)benzoic acid and the mixture heated under gentle reflux for 1.5 h. The excess thionyl chloride was removed under reduced pressure and the residue azeotroped with toluene (x2) to give crude 4-(2-(3-pyridyl)ethyl)benzoyl chloride hydrochloride as a solid. DCM (20 ml) was added and the resultant suspension treated with L-leucine ethyl ester hydrochloride (733 mg, 3.73 mmol), triethylamine (5.2 ml, 37.3 mmol) and DMAP (30 mg). The mixture was stirred for 1 h at room temperature, diluted with DCM (50 ml) and the solution washed with water (2x20 ml) and saturated brine (20 ml). The combined organics were dried over anhydrous magnesium sulphate, filtered and evaporated to give a yellow oil. Column chromatography (silica: 4% methanol in DCM) gave N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucine ethyl ester (378 mg, 28%) as a yellow solid.

i.r. (Film) 3355, 2960, 1750, 1635, 1520, 1500, 1160 cm^{-1}

deltaH (400 MHz) 8.45 (1H, br d, J 3.7 Hz), 8.42 (1H, br s), 7.71 (2H, d, J 8.3 Hz), 7.43 (1H, dt, J 7.8, 1.9 Hz), 7.21 (1H, dd, J 7.7, 4.9 Hz), 7.18 (2H, d, J 8.3 Hz), 6.49 (1H, d, J 8.3 Hz), 4.83 (1H, m), 4.22 (2H, q, J 7.1 Hz), 2.96 (4H, m), 1.80-1.60 (3H, m), 1.29 (3H, t, J 7.1 Hz), 0.99 (3H, d, J 6.1 Hz), 0.97 (3H, d, J 6.3 Hz).

(d) N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucine ethyl ester

A solution of N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucine ethyl ester (378 mg mg, 1.03 mmol) in dry THF (16 ml) was added *via* cannula to a stirred suspension of sodium hydride (60% dispersion in oil: 10 mg, 0.25 mmol) in dry THF (3 ml) at room temperature under argon. The mixture was stirred for 0.5 h and dimethyl sulphate (107 µl, 1.13 mmol) added. The mixture was stirred for 1.5 h, saturated aqueous ammonium chloride added (5 ml), the mixture extracted with ethyl acetate (3x20 ml) and the organics washed with brine (10 ml). The combined organics were dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure. Column chromatography (silica: 30% hexane in ethyl acetate) gave N-methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucine ethyl ester (21 mg, 7%) as a pale yellow oil.

i.r. (Film) 2960, 1740, 1640, 1395, 1330, 1190 cm^{-1}

deltaH (400 MHz) 8.46-8.37 (2H, br m), 7.41 (1H, dt, J 7.7, 1.9 Hz), 7.35-7.08

(5H, br m), 5.34 (0.6H, m), 4.35 (0.4H, m), 4.21 (2H, br m), 3.00-2.82 (7H, m), 1.85-1.40 (3H, m), 1.29 (3H, t, J 7.1 Hz), 0.98 (3.6H, d, J 6.3 Hz), 0.85 (1.2H, d, J 5.0 Hz), 0.62 (1.2H, d, J 5.5 Hz).

deltaC (100.6 MHz) 173.37, 166.90, 149.36, 147.50, 147.44,, 144.81, 142.60, 136.74, 132.32, 128.82, 127.42, 123.60, 61.53, 51.33, 42.18, 37.24, 34.62, 25.12, 22.93, 22.28, 21.60, 14.27

Examples 131-139

The compounds of Examples 131-139 may be prepared by the method of Example 130 employing the appropriate amino acid derivative *in lieu* of L-leucine ethyl ester hydrochloride.

131. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucinyl ethyl ether

132. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucine i-propyl ester

133. N-Ethyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucine ethyl ester

134. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-norleucinyl ethyl ether

135. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-1-tetrahydrofuryl-3-methyl-butylamine

136. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-valine ethyl ester

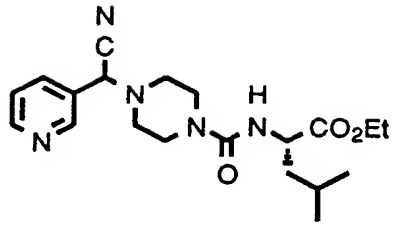
137. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-N'-methyl-L-tryptophan ethyl ester

138. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-O-benzyl-L-serine ethyl ester

139. N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-isoleucinyl ethyl ether

Example 140

N-4-(3-Pyridylcyanomethyl)piperazinecarbonyl-L-leucine ethyl ester



(a) 4-(3-Pyridylcyanomethyl)piperazine

A solution of nicotinaldehyde (5.1 ml, 54 mmol) in methanol (60 ml) was added to a stirred solution of piperazine (14.0 g, 160 mmol) and potassium cyanide (5.4 g, 83 mmol) in water (60 ml) and 1M phosphate buffer solution (pH 7.3: 60 ml). The reaction mixture was stirred for 48 h at ambient temperature and partitioned between water (80 ml) and ethyl acetate (2x100 ml). The organics were dried over anhydrous sodium sulphate, filtered and concentrated. Chromatography (silica gel: 2% methanol in DCM) gave 4-(3-pyridylcyanomethyl)piperazine (1.3 g, 12%) as a colourless oil.

delta$_H$ 8.71 (1H, d, J 2.3 Hz), 8.55 (1H, dd, J 4.8, 1.4 Hz), 7.79 (1H, dt, J 7.8, 2.1 Hz), 7.29 (1H, dd, J 7.7, 4.9 Hz), 4.81 (1H, s), 2.84 (4H, m), 2.48 (4H, m), 1.68 (1H, br s).

delta$_C$ 150.03, 149.22, 135.27, 128.74, 123.20, 114.16, 60.44, 50.87, 45.52.

(b) Dipyrid-2-ylcarbonate

Triethylamine (10.5 ml, 75 mmol) was added slowly to a solution of triphosgene (3.0 g, 10 mmol) and 2-hydroxypyridine (5.7 g, 60 mmol) in dry DCM (500 ml) at 0°C under argon. The mixture was allowed to warm to room temperature and was stirred overnight. The solvent was removed under reduced pressure and the residue taken up in ethyl acetate (500 ml), washed with saturated aqueous sodium hydrogen carbonate (2x150 ml) and brine (200 ml), dried over anhydrous sodium sulphate filtered and concentrated to give an orange oil. Crystallisation from ethyl acetate/hexane gave dipyrid-2-ylcarbonate as an off-white crystalline solid (3.70 g, 57%).

delta$_H$ 8.42 (2H, dd, J 4.8, 1.1 Hz), 7.83 (2H, ddd, J 7.8, 7.7, 1.8 Hz), 7.30-7.23 (4H, m).

(c) N-4-(3-Pyridylcyanomethyl)piperazinecarbonyl-L-leucine ethyl ester

Dipyrid-2-ylcarbonate (234 mg, 1.1 mmol) was added to a stirred solution of triethylamine (100 µl, 1.1 mmol) and 4-(3-pyridylcyanomethyl)piperazine (140 mg, 0.7 mmol) in dry DCM (6 ml) at room temperature under argon. The mixture was stirred overnight, DCM (50 ml) added and the solution washed with saturated aqueous sodium hydrogen carbonate and brine. The organics were dried over anhydrous sodium sulphate, filtered and concentrated to give crude 4-(3-pyridylcyanomethyl)piperazinepyrid-2-ylcarbonate as a colourless oil. Dry DCM (2 ml) was added and the resultant solution transfered *via* cannula to a stirred mixture of L-leucine ethyl ester hydrochloride (160 mg, 0.8 mmol) and triethylamine (200 µl, 2.2 mmol) in dry DCM (10 ml) at room temperature under argon. The mixture was stirred overnight, DCM (50 ml) added and the solution washed with 10% aqueous citric acid. The organics were concentrated under reduced pressure and the residue partitioned between ethyl acetate and saturated aqueous sodium hydrogen carbonate. The organic phase was washed with brine, dried over anhydrous sodium sulphate, filtered and evaporated to give a yellow foam. Chromatography (silica: 3% methanol in DCM) gave N-4-(3-pyridylcyanomethyl)piperazinecarbonyl-L-leucine ethyl ester (62 mg, 23%) as a colourless oil.

delta$_H$ 8.70 (1H, d, J 2.3 Hz), 8.54 (1H, dd, J 4.8, 1.4 Hz), 7.77 (1H, dt, J 7.8, 2.1 Hz), 7.30 (1H, dd, J 7.7, 4.9 Hz), 4.81 (1H, s), 4.60-4.45 (1H, m), 4.10 (2H, q, J 7.1 Hz), 3.60-3.30 (4H, m), 2.45-2.30 (4H, m), 1.67-1.38 (3H, m), 1.21 (3H, t, J 7.5 Hz), 0.87 (3H, d, J 6.1 Hz), 0.84 (3H, d, J 6.3 Hz).

Examples 141-143

The compounds of Examples 141-143 may be prepared by the method of Example 140 employing the appropriate amino acid derivative *in lieu* of L-leucine ethyl ester hydrochloride. The tertiary carbamates may be prepared by alkylation of the corresponding secondary amides or carbamates by the method of Example 130 Step (d).

141. N-4-(3-Pyridylcyanomethyl)piperazinecarbonyl-L-leucine ethyl ester

142. N-Methyl-N-4-(3-pyridylcyanomethyl)piperazinecarbonyl-L-leucine ethyl ester

143. N-Methyl-N-4-(3-pyridylcyanomethyl)piperazinecarbonyl-L-leucinyl ethyl ether

Examples 144-149

The compounds of Examples 144-149 may be prepared by the method of Example 140 Step (a) employing the appropriate N-alkyl-N-4-piperidine-carbonyl amino acid derivative *in lieu* of piperazine.

144. N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-leucine ethyl ester

145. N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-leucinyl ethyl ether

146. N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-leucine propyl ester

147. N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-isoleucine ethyl ester

148. N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-phenylalanine ethyl ester

149. N-Ethyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-leucinyl ethyl ether

Example 150

Inhibition of [^{3}H]-PAF Receptor Binding

The inhibition of [^{3}H]-PAF binding to human platelet plasma membrane by compounds of general formula I was determined by isotopic labelling and filtration techniques. Platelet concentrates were obtained from a hospital blood bank. These platelet concentrates (500-2500 ml.) were centrifuged at 800 rpm for 10 minutes in a SORVALL RC3B centrifuge to remove the red blood cells present. (The word SORVALL is a trade mark.) The supernatant was subsequently centrifuged at 3,000 rpm in a SORVALL RC3B centrifuge to pellet the platelets present. The platelet rich pellets were resuspended in a minimum volume of buffer (150 mM NaCl, 10 mM Tris, 2 mM EDTA, pH 7.5) and layered

onto Ficoll-Paque gradients, 9 ml platelet concentrate to 2 ml Ficoll, and centrifuged at 1,900 rpm for 15 minutes in a SORVALL RT6000 centrifuge. This step removes the residual red blood cells and other nonspecific material such as lymphocytes from the preparation. The platelets which form a band between the plasma and the Ficoll were removed, resuspended in the above buffer and centrifuged at 3,000 rpm for 10 minutes in a SORVALL RT6000 centrifuge. The pelleted platelets were resuspended in buffer (10 mM Tris, 5 mM $MgCl_2$, 2 mM EDTA, pH 7.0), snap freezed in liquid N_2 and allowed to thaw slowly at room temperature in order to lyse the platelets. The latter step was repeated at least 3 times to ensure proper lysis. The lysed platelets were centrifuged at 3,000 rpm for 10 minutes in a SORVALL RT6000 centrifuge and resuspended in buffer. The latter step was repeated twice in order to remove any cytoplasmic proteins which may hydrolyse the platelet activating factor (PAF) receptor. The prepared platelet membranes may be stored at -70°C. After thawing the prepared membranes were centrifuged in a SORVALL RT6000 at 3,000 rpm for 10 minutes and resuspended in assay buffer.

The assay was conducted by preparing a series of Tris-buffered solutions of the selected antagonist of predetermined concentrations. Each of these solutions contained [^{3}H]-PAF (0.5 nM; 1-*O*-[^{3}H]octadecyl-2-acetyl-*sn*-glycero-3-phosphoryl choline with a specific activity of 132 Ci/mmol), unlabelled PAF (1000 nM), a known amount of the test antagonist, and a sufficient amount of Tris-buffer solution (10 mM Tris, 5 mM $MgCl_2$, pH 7.0, 0.25% BSA) to make the final volume 1 ml. Incubation was initiated by the addition of 100 µg of the isolated membrane fraction to each of the above solutions at 0°C. Two control samples, one (C1) which contained all the ingredients described above except the antagonist and the other (C2) contains C1 plus a 1000-fold excess of unlabelled PAF, were also prepared and incubated simultaneously with the test samples. After 1 hour incubation, each solution was filtered rapidly under vacuo through a WHATMAN GF/C glass fibre filter in order to separate unbound PAF from bound PAF. (The word WHATMAN is a trade mark.) The residue in each case was rapidly washed 4 times with 5 ml cold (4°C) Tris-buffer solution. Each washed residue was dried under vacuum on a sampling manifold and placed into vials containing 20 ml of OPTIPHASE MP scintillation fluid and the radioactivity counted in a liquid scintillation counter. (The word OPTIPHASE is a trade mark.) Defining the counts for total binding with antagonist from a test sample as "TBA"; the counts for total binding from the control sample C1 as "TB"; and the counts for nonspecific binding from the control sample C2 as "NSB", the percent inhibition

of each test antagonist can be determined by the following equation:

%Inhibition = [(TB-TBA)/SB]x100

where the specific binding SB = TB-NSB

Table 1 lists results from this assay for inhibition of [^{3}H]-PAF receptor binding for illustrative examples of the compounds of this invention.

Table 1: Results for inhibition of [^{3}H]-PAF receptor binding

Example	Inhibition of [^{3}H]-PAF binding IC_{50} nM
1B	15
11B	3
98B	1

Example 151

Inhibition of PAF-Induced Hypotension in the Rat

The activity of the compounds of general formula I is also demonstrated *in vivo* by their ability to reverse the hypotension caused by an infusion of PAF in rats. Male Sprague-Dawley rats (300-350 g) were anaesthetised with a mixture of sodium pentobarbitone, 22.5 mg/kg and thiopental 62.5 mg/kg. Through a midline incision in the neck, the trachea was cannulated and the animals breathed spontaneously. A carotid artery was cannulated for the measurement of blood pressure and this signal was used to trigger a rate meter to measure heart rate. Both jugular veins were cannulated: one for the infusion of PAF and the other for the bolus administration of test compounds.

PAF, 100 ng/kg/min was infused *i.v.* until a sustained fall in mean blood pressure of 50 mmHg was achieved. Test compounds were administered *i.v.* as a bolus and resulted in a dose dependent reversal of the PAF induced hypotension. The peak of this reversal was measured and the dose to cause a 50% reversal of the

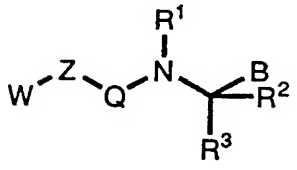
hypotensive PAF response (ED_{50}) calculated by straight line interpolation and the results are presented in Table 2.

Table 2: Results for inhibition of PAF-induced hypotension in the rat

Example	ED_{50} (μg/kg *i.v.*)
11B	30.5
33B	1.8

CLAIMS

1. A compound of general formula I:



I

wherein:

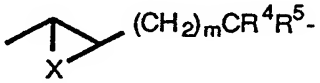
W represents pyrid-3-yl, benzimidazol-1-yl, imidazo[4,5-c]pyridin-1-yl, imidazo[4,5-c]pyridin-3-yl and imidazo[4,5-c]pyridin-5-yl optionally substituted with one or more -C_1-C_6 alkyl substituents;

Z represents:

a) a divalent alkanediyl, alkenediyl or alkynediyl group from 2 to 12 carbon atoms which may be a straight or branched-chain provided that, when Z represents a branched chain at least two carbon atoms separate W from the group Q, wherein the said group is either unsubstituted or substituted by one or more substituents selected from hydroxy, -OC_1-C_6 alkyl, -SC_1-C_6 alkyl and halo; or

b) a -$(CH_2)_qU(CH_2)_r$- group, optionally substituted by one or more substituents selected from hydroxy, -OC_1-C_6 alkyl, halo and nitrile, wherein q is an integer from 0-3, r is an integer from 0-3 and U is -O-, -S- or a furandiyl, tetrahydrofurandiyl, thiophenediyl, tetrahydrothiophenediyl, thiazolediyl, tetrahydrothiazolediyl, piperazinediyl, piperidinediyl, cyclopentanediyl, cyclohexanediyl, cycloheptenediyl or benzenediyl group (provided that, when U is a 1,4-benzenediyl group q is not an integer of 1); or

c) a

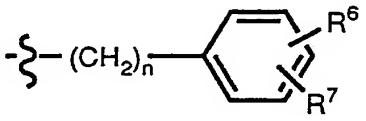


group wherein m is an integer from 0-3, X is -O-, -S- or -CH_2- and each of R^4 and R^5 is independently hydrogen or -C_1-C_6 alkyl;

Q represents a carbonyl, thiocarbonyl or sulphonyl group or a bond

R^1 represents hydrogen, -C_1-C_6 alkyl, -C_2-C_6 alkenyl, -C_2-C_6 alkynyl, -COC_1-C_6 alkyl, -CO_2C_1-C_6 alkyl, -(CO_2C_1-C_6 alkyl)phenyl, -(C_1-C_6 alkyl)CO_2C_1-C_6 alkyl, -(C_1-C_6 alkyl)phenyl, -C_3-C_8 cycloalkyl, -C_4-C_8 cycloalkenyl or phenyl optionally substituted by one or more substituents selected from -C_1-C_6 alkyl, -OC_1-C_6 alkyl, halogen, -CF_3 and -CN;

R^2 represents hydrogen, halogen, -C_1-C_6 alkyl optionally substituted by one or more halogen atoms, -C_2-C_6 alkenyl, -C_2-C_6 alkynyl, -(C_1-C_6 alkyl)CO_2C_1-C_6 alkyl, -(C_1-C_6 alkyl)SC_1-C_6 alkyl, -(C_1-C_6 alkyl)OC_1-C_6 alkyl, -(C_1-C_6 alkyl)N(C_1-C_6 alkyl)2, -C_3-C_8 cycloalkyl, -C_4-C_8 cycloalkenyl, -(C_1-C_6 alkyl)C_3-C_8 cycloalkyl, -(C_1-C_6 alkyl)C_4-C_8 cycloalkenyl, -(C_1-C_6 alkyl)OC_3-C_8 cycloalkyl, -(C_1-C_6 alkyl)OC_4-C_8 cycloalkenyl, -(C_1-C_6 alkyl)SC_3-C_8 cycloalkyl, -(C_1-C_6 alkyl)SC_4-C_8 cycloalkenyl, a side chain of a naturally occurring amino acid, a group -D or -(C_1-C_6 alkyl)OD wherein D is a group



D

wherein n is an integer from 0 to 3, and

each of R^6 and R^7 is independently hydrogen, -C_1-C_6 alkyl, -C_2-C_6 alkenyl, -C_2-C_6 alkynyl, halogen, -CN, -CO_2H, -CO_2C_1-C_6 alkyl, -$CONH_2$, -$CONHC_1$-C_6 alkyl, -$CON(C_1$-C_6 alkyl)2, -CHO, -CH_2OH, -CF_3, -OC_1-C_6 alkyl, -SC_1-C_6 alkyl, -SOC_1-C_6 alkyl, -SO_2C_1-C_6 alkyl, -NH_2 or -NHCOMe; or

R^1 together with R^2 and the atoms to which they are attached form a 5 to 8 membered nitrogen-containing heterocyclic ring;

R^3 represents hydrogen or halogen;

B represents:

a) a -VR^8 group wherein V is -C(=O)-, -C(=O)O-, -CH_2O-, -CH_2OC(=O)-, -C(=S)-, -CH_2OC(=O)NH-, -C(=S)O-, -CH_2S-, -C(=O)$NHSO_2$- or

-$SO_2NHC(=O)$-; and

R^8 is hydrogen, -C_1-C_{18} alkyl, -C_2-C_{18} alkenyl, -C_2-C_{18} alkynyl, -(C_1-C_6 alkyl)OC_1-C_6 alkyl, -(C_1-C_6 alkyl)SC_1-C_6 alkyl, -(C_1-C_6 alkyl)$O(C_1$-C_6 alkyl)OC_1-C_6 alkyl, -C_3-C_8 cycloalkyl, -C_4-C_8 cycloalkenyl or pyridyl, (any of which may optionally be substituted with one or more substituents selected from halogen, hydroxyl, nitro, nitrile or carboxyl), -C_1-C_4 perfluoroalkyl, a group -D as defined above or a -(C_1-C_6 alkyl)OD group wherein D is as defined above;

b) a -$CH_2NR^9R^{10}$ group or a -$CONR^9R^{10}$ group wherein each of R^9 and R^{10} is independently hydrogen, -C_1-C_{18} alkyl, -C_2-C_{18} alkenyl, -C_2-C_{18} alkynyl, -C_3-C_8 cycloalkyl, -C_4-C_8 cycloalkenyl, pyridyl (any of which may optionally be substituted with one or more substituents selected from halogen, hydroxyl, nitro, nitrile or carboxyl) or a group -D as defined above or R^9 and R^{10} together with the nitrogen atom to which they are attached form a 5 to 8 membered nitrogen-containing heterocyclic ring;

c) a group Y where Y is a 5- or 6-membered heterocyclic ring containing one or more heteroatoms selected from nitrogen, oxygen and sulphur and the ring may be optionally substituted with one or more substituents selected from -C_1-C_6 alkyl, -OC_1-C_6 alkoxy, halogen, -CF_3 and -CN; or

d) a group -CH_2-Y or C(=O)NHY; where Y is as defined above;

or a pharmaceutically or veterinarily acceptable acid addition salt or hydrate thereof.

2. A compound as claimed in Claim 1 wherein W represents pyrid-3-yl, 2-methylbenzimidazol-1-yl, 2-methylimidazo[4,5-c]pyridin-1-yl, 2-methylimidazo[4,5-c]pyridin-3-yl and 2-methylimidazo[4,5-c]pyridin-5-yl;

3. A compound as claimed in Claim 2, wherein Z represents:

a) an alkanediyl having from 3 to 11 carbon atoms group, an alkenediyl group or an alkynediyl group, or;

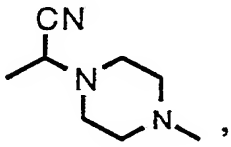
b) a -$(CH_2)_qU(CH_2)_r$- group, optionally substituted by nitrile, wherein;

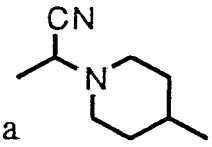
U represents -O-, -S- or a tetrahydrofurandiyl, furandiyl, a thiophenediyl, a piperidinediyl or a benzenediyl group;

q represents an integer of 0, 1, or 2 (provided that, when U is a 1,4-benzenediyl group q is not an integer of 1); and

r represents an integer of 0.

4. A compound as claimed in Claim 3, wherein Z represents a propylene, 2-hydroxypropylene, 1-methylpropylene, 1,1-dimethylpropylene, butylene, 1-methylbutylene, 1,1-dimethylbutylene, 3-hydroxybutylene, pentylene, 1-methylpentylene, 1,1-dimethylpentylene, 4-hydroxypenylene, 4-methoxypentylene, hexylene, 1,1-dimethylhexylene, heptylene, 1-methylheptylene, 1,1-dimethylheptylene, octylene, 1,1-dimethyloctylene, nonylene, decylene, undecylene a



, a


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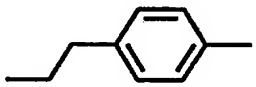
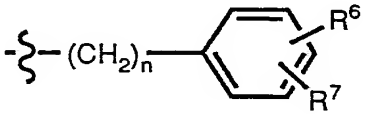
or a

group.

5. A compound as claimed in any one of Claims 1 to 4, wherein Q represents a carbonyl or sulphonyl group.

6. A compound as claimed in any one of Claims 1 to 5, wherein R^1 represents a hydrogen atom, a -C_1-C_6 alkyl group, a -C_2-C_6 alkenyl group, or a -(C_1-C_6 alkyl)CO_2C_1-C_6 alkyl group.

7. A compound as claimed in any one of Claims 1 to 6, wherein R^2 represents a -C_1-C_6 alkyl group, a -C_2-C_6 alkenyl group, a -(C_1-C_6 alkyl)SC_1-C_6 alkyl group, the side chain of a naturally occurring amino acid, a group -D or a -(C_1-C_6 alkyl)OD group;



D

wherein n represents an integer of 0 or 1, R^6 represents a hydrogen atom or a -OC_1-C_6 alkyl group and R^7 represents a hydrogen atom.

8. A compound as claimed in any one of Claims 1 to 7, wherein R^3 represents a hydrogen atom.

9. A compound as claimed in Claim 7, wherein R^3 represents the side chain of the amino acid leucine.

10. A compound as claimed in any one of Claims 1 to 9, wherein B represents a $-VR^8$ group, a $-CONR^9R^{10}$ group or a group Y.

11. A compound as claimed in Claim 10 wherein V represents a -C(=O)O- group, a $-CH_2OC(=O)-$ group, a $-CH_2O-$ group, a $-CH_2OC(=O)-$ group or a $-CH_2OC(=O)NH-$ group.

12. A compound as claimed in Claim 10 wherein Y represents a a pyrazinyl group or a oxadiazolyl group.

12. A compound as claimed in Claim 10, or Claim 11, wherein R^8 represents a hydrogen atom, $-C_1-C_{18}$ alkyl group, a $-C_2-C_{18}$ alkenyl group, a $-(C_1-C_6$ alkyl)$O(C_1-C_6$ alkyl)OC_1-C_6 alkyl group, a pyridyl group, a group D or a $-(C_1-C_6$ alkyl)OD group.

13. A compound as claimed in Claim 10 wherein R^9 is a pyridyl group and R^{10} is a hydrogen atom.

14. N-6-(2-Methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-leucine ethyl ester,
N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine ethyl ester,
N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-D-leucine ethyl ester,
N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-phenylalanine ethyl ester,
N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine propyl ester,
N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-norleucine ethyl ester,
N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-O-benzyl-L-serine methyl ester,
N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2-methylhexanoyl-L-leucine ethyl ester,
N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethylhexanoyl-L-leucine ethyl ester,
N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)-5-hydroxyhexanoyl-L-leucine ethyl

ester,
N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine hexadecyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-3-yl)butanoyl-L-leucine ethyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine ethyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-5-yl)butanoyl-L-leucine ethyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine methyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-O-methyl-L-tyrosine ethyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-methionine ethyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-norleucine n-butyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethylbutanoyl-L-leucine ethyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)-3-hydroxybutanoyl-L-leucine ethyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-valine ethyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine hexyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine decyl ester,
N-3-(2-Methylbenzimidazol-1-yl)propylsulphonyl-L-leucine ethyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-alanine ethyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-isoleucine ethyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-norleucine ethyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-methionine ethyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine i-propyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine pentyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine octyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine dodecyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine pentadecyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine hexadecyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine octadecyl ester,
N-4-(2-Methylimidazo[4,5-c]pyridin-3-yl)propylsulphonyl-L-leucinyl ethyl ether,

N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl ethyl ether,
N-4-(2-methylimidazo[4,5-c]pyridin-5-yl)propylsulphonyl-L-leucinyl ethyl ether,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl methyl ether,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl octyl ether,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl hexadecyl ether,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl benzyl ether,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl propionate,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl octadecanoate,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl N'-ethyl carbamate,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl N'-benzyl carbamate,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl N'-2-pyridylcarbamate,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl N-octadecylcarbamate,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-1-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine,
N-5-(2-Methylimidazo[4,5-c]pyridin-3-yl)pentanoyl-L-leucine ethyl ester,
N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucine ethyl ester,
N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucine i-propyl ester,
N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-O-methyl-L-tyrosine ethyl ester,
N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-D,L-allylglycine ethyl ester,
N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-norleucine allyl ester,
N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2-methylpentanoyl-L-leucinyl ethyl ether,
N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethylpentanoyl-L-leucine 2-benzoxyethylethyl ester,
N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)-3-hydroxypentanoyl-L-leucine 2-(2-ethoxyethoxy)ethyl ester,
N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-1-(3-methyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine,
N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-1-(6-ethylpyrazin-2-yl)-3-

methylbutylamine,
N-5-(2-Methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucinyl N'-ethyl-carbamate,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-leucine ethyl ester,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine ethyl ester,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-leucine ethyl ester,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-isoleucine allyl ester,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-isoleucine allyl ester,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-isoleucine allyl ester,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-leucinyl ethyl ether,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucinyl ethyl ether,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-leucinyl ethyl ether,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucinyl hexadecyl ether,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-phenyl-alaninyl ethyl ether,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucinyl 4-methoxybenzyl ether,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-norleucinyl ethyl ether,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-O-benzyl-L-serinyl ethyl ether,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)-2-methylhexanoyl-L-leucinyl ethyl ether,
N-Ethoxycarbonyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucinyl ethyl ether,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)-5-methoxyhexanoyl-L-leucinyl ethyl ether,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-1-(3-ethyl-1,2,4-

oxadiazol-5-yl)-3-methylbutylamine,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine ethyl ester,
N-Allyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine i-propyl ester,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucinyl ethyl ether,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)-2-methylbutanoyl-L-leucinyl ethyl ether,
N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucine ethyl ester,
N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucinyl ethyl ether,
N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)-2-methylpentanoyl-L-leucinyl ethyl ether,
N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentanoyl-L-leucinyl hexadecyl ether,
N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine ethyl ester,
N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucine i-propyl ester,
N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl ethyl ether,
N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-leucinyl hexadecyl ester,
N-Methyl-N-3-(2-methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-1-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butylsulphonyl-L-leucine ethyl ester,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butylsulphonyl-L-leucinyl ethyl ether,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)butylsulphonyl-L-leucinyl heptadecyl ether,
N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentylsulphonyl-L-leucine ethyl ester,
N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentylsulphonyl-L-leucine i-propyl ester,
N-Methyl-N-5-(2-methylimidazo[4,5-c]pyridin-1-yl)pentylsulphonyl-L-leucinyl

ethyl ether,
N-8-(2-Methylimidazo[4,5-c]pyridin-3-yl)octanoyl-L-leucine ethyl ester,
N-8-(2-Methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucine ethyl ester,
N-8-(2-Methylimidazo[4,5-c]pyridin-5-yl)octanoyl-L-leucine ethyl ester,
N-8-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2-methyloctanoyl-L-leucine ethyl ester,
N-8-(2-Methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethyloctanoyl-L-phenylalanine ethyl ester,
N-Methyl-N-8-(2-methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucine i-propyl ester,
N-Methyl-N-8-(2-methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucinyl ethyl ether,
N-Methyl-N-8-(2-methylimidazo[4,5-c]pyridin-1-yl)octanoyl-1-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine,
N-7-(2-Methylimidazo[4,5-c]pyridin-1-yl)heptanoyl-L-leucine ethyl ester,
N-Methyl-N-7-(2-methylimidazo[4,5-c]pyridin-1-yl)heptanoyl-L-leucinyl ethyl ether,
N-Methyl-N-7-(2-methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethylheptanoyl-L-leucinyl ethyl ether,
N-Methyl-N-7-(2-methylimidazo[4,5-c]pyridin-1-yl)heptanoyl-L-leucinyl N'-hexadecylcarbamate,
N-11-(2-Methylbenzimidazol-1-yl)undecanoyl-L-leucine ethyl ester,
N-11-(2-Methylimidazo[4,5-c]pyridin-3-yl)undecanoyl-L-leucine ethyl ester,
N-11-(2-Methylimidazo[4,5-c]pyridin-1-yl)undecanoyl-L-leucine ethyl ester,
N-9-(2-Methylimidazo[4,5-c]pyridin-1-yl)nonanoyl-L-leucine ethyl ester,
N-Methyl-N-9-(2-methylimidazo[4,5-c]pyridin-1-yl)nonanoyl-L-leucine i-propyl ester,
N-Methyl-N-9-(2-methylimidazo[4,5-c]pyridin-1-yl)nonanoyl-L-leucinyl ethyl ether,
N-Methyl-N-9-(2-methylimidazo[4,5-c]pyridin-1-yl)-2,2-dimethylnonanoyl-L-leucinyl ethyl ether,
N-Methyl-N-10-(2-methylimidazo[4,5-c]pyridin-1-yl)decanoyl-L-leucinyl ethyl ester,
N-Methyl-N-10-(2-methylimidazo[4,5-c]pyridin-1-yl)decanoyl-L-leucine ethyl ester,
N-Methyl-N-11-(2-methylimidazo[4,5-c]pyridin-1-yl)undecanoyl-L-leucine ethyl ester,
N-Methyl-N-11-(2-methylimidazo[4,5-c]pyridin-1-yl)undecanoyl-L-leucinyl ethyl ether,

N-Methyl-N-12-(2-methylimidazo[4,5-c]pyridin-1-yl)dodecanoyl-L-leucinyl ethyl ether,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-D-leucine ethyl ester,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-phenylalanine ethyl ester,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-5-yl)hexanoyl-L-leucine,
N-Methyl-N-6-(2-methylimidazo[4,5-c]pyridin-3-yl)hexanoyl-L-isoleucine,
N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-leucine,
N-6-(2-Methylimidazo[4,5-c]pyridin-1-yl)hexanoyl-L-phenylalanine,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)butanoyl-L-leucine,
N-4-(2-Methylimidazo[4,5-c]pyridin-1-yl)propylsulphonyl-L-methionine,
N-8-(2-Methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucine,
N-Methyl-N-8-(2-methylimidazo[4,5-c]pyridin-1-yl)octanoyl-L-leucine,
N-Methyl-N-11-(2-methylimidazo[4,5-c]pyridin-1-yl)undecanoyl-L-leucine,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine ethyl ester,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucinyl ethyl ether,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-phenylalanine ethyl ester,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine n-butyl ester,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-isoleucine ethyl ester,
N-Ethyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine ethyl ester,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucine 2-pyridyl amide,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucinyl N'-ethyl-carbamate,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-L-leucinyl ethanoate,
N-Methyl-N-4-(2-methylimidazo[4,5-c]pyridin-1-yl)benzoyl-1-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-methylbutylamine,
N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucine ethyl ester,
N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucinyl ethyl ether,
N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucine i-propyl ester,
N-Ethyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-leucine ethyl ester,

N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-norleucinyl ethyl ether,
N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-1-tetrahydrofuryl-3-methylbutyl-amine,
N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-valine ethyl ester,
N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-N'-methyl-L-tryptophan ethyl ester,
N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-O-benzyl-L-serine ethyl ester,
N-Methyl-N-4-(2-(3-pyridyl)ethyl)benzoyl-L-isoleucinyl ethyl ether,
N-4-(3-Pyridylcyanomethyl)piperazinecarbonyl-L-leucine ethyl ester,
N-4-(3-Pyridylcyanomethyl)piperazinecarbonyl-L-leucine ethyl ester,
N-Methyl-N-4-(3-pyridylcyanomethyl)piperazinecarbonyl-L-leucine ethyl ester,
N-Methyl-N-4-(3-pyridylcyanomethyl)piperazinecarbonyl-L-leucinyl ethyl ether,
N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-leucine ethyl ester,
N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-leucinyl ethyl ether,
N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-leucine propyl ester,
N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-isoleucine ethyl ester,
N-Methyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-phenylalanine ethyl ester,
N-Ethyl-N-4-(3-pyridylcyanomethyl)piperidinecarbonyl-L-leucinyl ethyl ether;

or a salt of such a compound.

15. A compound as claimed in any one of Claims 1 to 14 for use in human or veterinary medicine.

16. A compound as claimed in Claim 15 for use in the treatment or prophylaxis of diseases and conditions mediated by PAF.

17. The use of a compound as claimed in any one of Claims 1 to 14 in the preparation of an agent for the treatment or prophylaxis of diseases or conditions mediated by platelet activating factor.

18. A pharmaceutical or veterinary composition comprising a compound as claimed in any one of Claims 1 to 14 and a pharmaceutically and/or veterinarily acceptable carrier.

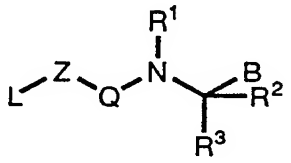
19. A process for preparing a compound of general formula I as defined in

Claim 1, the process comprising:

(a) treating a nitrogen heterocycle represented by general formula II

W-H II

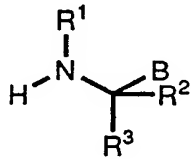
wherein W is as defined in general formula I, with a suitable base (e.g. sodium hydride, potassium hydride or sodium bis(trimethylsilyl)amide), followed by a compound of general formula III



III

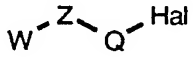
wherein Z, Q, R^1, R^2, R^3 and B are as defined in general formula I, and L is a leaving group such as chloro, bromo, iodo, methanesulphonyloxy, p-toluenesulphonyloxy or trifluoromethanesulphonyloxy;

(b) treating an amine represented by general formula IV



VI

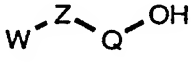
wherein R^1, R^2, R^3, and B are as defined in general formula I, with a suitable base in an aprotic solvent followed by a halo derivative of general formula V



V

wherein W, Z and Q are as defined in general formula I and Hal is a halide such as fluoro, chloro, bromo or iodo;

(c) treating an amine of general formula IV with a derivative of general formula VI

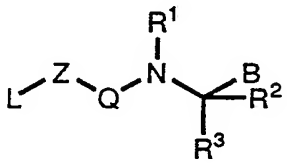


VI

wherein W and Z are as defined in general formula I and Q represents a -C(=O)- group, in the presence of a coupling reagent; and

(d) optionally after step (a), step (b) or step (c) converting, in one or a plurality of steps, a compound of general formula I into another compound of general formula I.

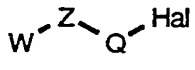
20. A compound of general formula III



III

wherein Z, Q, R^1, R^2, R^3 and B are as defined in general formula I, and L is a leaving group such as chloro, bromo, iodo, methanesulphonyloxy, p-toluenesulphonyloxy or trifluoromethanesulphonyloxy.

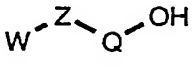
21. A compound of general formula V



V

wherein W, Z and Q are as defined in general formula I and Hal is a halide such as fluoro, chloro, bromo or iodo.

22. A compound of general formula VI



VI

wherein W, Z and Q are as defined in general formula I.

23. A method for the treatment or prophylaxis of diseases or physiological conditions of humans or mamalian animals mediated by platelet activating factor, comprising administering to the patient an amount of a compound as claimed in any of claims 1 to 14 effective to antagonise the effects of platelet activating factor on target cells responsible for such diseases or physiological conditions.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00009

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07D235/08; C07D213/56; C07D471/04; A61K31/435
A61K31/415; C07C229/10; C07C311/04; //(C07D471/04, ./.

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07D ; C07C ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	WO,A,9 117 162 (PFIZER) 14 November 1991 see page 1, line 1 - line 6; claim 1	1,15,16
X	WO,A,9 100 725 (ABBOTT) 24 January 1991 see claims 1,8	1,15
A	WO,A,9 009 997 (BRITISH TECHNOLOGY GROUP) 7 September 1990 see claims 1,37	1,15,16
X	WO,A,9 012 015 (PFIZER) 18 October 1990 see page 19, paragraph 2 - page 20, paragraph 1	21,22

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° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19 APRIL 1993	11. 05. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	ALFARO FAUS I.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00009

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: 235:00; 221:00)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]

* Special categories of cited documents: [10]

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	CHEMICAL ABSTRACTS, vol. 80, 1974, Columbus, Ohio, US; abstract no. 60217n, M. SEMONSKY ET AL. 'N-(delta-(6-Purinylthio)valeryl)amino acids and derivatives' page 385 ; see abstract & CS,A,150 002 15 August 1973	20
X	TETRAHEDRON, (INCL. TETRAHEDRON REPORTS) vol. 28, 1972, OXFORD GB pages 2539 - 2544 G. SNATKKE ET AL. 'Circular dichroism. LIII. Chiroptical properties of amino acid sultam derivatives' see pages 2540 and 2543, compounds 1e,2f,3f and 4f	20
P,A	WO,A,9 203 422 (BRITISH BIO-TECHNOLOGY) 5 March 1992 see claims 1,19	1,15,16
P,A	WO,A,9 203 423 (BRITISH BIO-TECHNOLOGY) 5 March 1992 see claims 1,21	1,15,16
P,A	WO,A,9 218 503 (BRITISH BIO-TECHNOLOGY) 29 October 1992 see claims 1,19,20	1,15,16

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9300009
SA 68717

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 19/04/93
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9117162	14-11-91	EP-A-	0530207	10-03-93
WO-A-9100725	24-01-91	CA-A-	2062755	08-01-91
		EP-A-	0480969	22-04-92
		JP-T-	4506660	19-11-92
WO-A-9009997	07-09-90	AU-A-	5162690	26-09-90
		EP-A-	0468971	05-02-92
		JP-T-	4505156	10-09-92
WO-A-9012015	18-10-90	EP-A-	0467895	29-01-92
		JP-T-	4503673	02-07-92
WO-A-9203422	05-03-92	AU-A-	8421691	17-03-92
		AU-A-	8426891	17-03-92
		WO-A-	9203423	05-03-92
		US-A-	5180723	19-01-93
WO-A-9203423	05-03-92	AU-A-	8421691	17-03-92
		AU-A-	8426891	17-03-92
		WO-A-	9203422	05-03-92
		US-A-	5180723	19-01-93
WO-A-9218503	29-10-92	US-A-	5180724	19-01-93